PREVALENCE OF SEROPOSITIVITY AGAINST HEPATITIS B VIRUS IN CHILDREN AGED 10 TO 15 YEARS WHO HAVE BEEN IMMUNIZED AGAINST HEPATITIS B IN INFANCY

Dissertation Submitted to

THE TAMILNADU DR.M.G.R. MEDICAL UNIVERSITY
CHENNAI, TAMILNADU

In fulfillment of the regulations for the award of the degree

M.D.(PEDIATRICS)

DEPARTMENT OF PEDIATRICS

PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH

COIMBATORE, TAMILNADU

APRIL 2016

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M.D. (PEDIATRICS)

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THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY
CHENNAI, TAMILNADU

APRIL 2016

DECLARATION

I hereby declare that this dissertation entitled **"PREVALENCE OF SEROPOSITIVITY AGAINST HEPATITIS B VIRUS IN CHILDREN AGED 10 TO 15 YEARS WHO HAVE BEEN IMMUNIZED AGAINST HEPATITIS B IN INFANCY"** was prepared by me under the guidance and supervision of **Dr.A.M.VIJAYALAKSHMI**, Professor of Pediatrics, PSGIMS&R, Coimbatore.

This dissertation is submitted to The Tamilnadu Dr. M.G.R Medical University, Chennai in fulfillment of the university regulations for the award of MD degree in Pediatrics. This dissertation has not been submitted elsewhere for the award of any other Degree or Diploma.

Dr.KUMARAGURU.R

CERTIFICATE

This is to certify that the thesis entitled **"PREVALENCE OF SEROPOSITIVITY AGAINST HEPATITIS B VIRUS IN CHILDREN AGED 10 TO 15 YEARS WHO HAVE BEEN IMMUNIZED AGAINST HEPATITIS B IN INFANCY"** is the bonafide work of **Dr.KUMARAGURU.R**, done under my guidance and supervision in the Department of Pediatrics, PSG IMS&R, Coimbatore in fulfillment of the regulations laid down by The Tamilnadu Dr. M.G.R. Medical University for the award of MD degree in Pediatrics.

Dr. A.M.VIJAYALAKSHMI
Professor
Department of Pediatrics
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CERTIFICATE

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August 4, 2014

To
Dr R Kumaraguru
Postgraduate
Department of Paediatrics
PSG IMS & R
Coimbatore

The Institutional Human Ethics Committee, PSG IMS & R, Coimbatore -4, has reviewed your proposal on 18th July, 2014 in its expedited review meeting held at IHEC Secretariat, PSG IMS&R, between 10.00 am and 11.00 am, and discussed your study proposal entitled:

"Prevalence of seropositivity against hepatitis B virus in children aged 10 to 15 years who have been immunized against hepatitis B in infancy"

The following documents were received for review:

1. Duly filled application form
2. Proposal
3. Assent form
4. Parental consent form
5. Data collection tool
6. CV
7. Budget

After due consideration, the Committee has decided to approve the study.

The members who attended the meetings at which your study proposal was discussed are as follows:

Name	Qualification	Responsibility in IHEC	Gender	Affiliation to the Institution Yes/No	Present at the meeting Yes/No
Dr P Sathyan	DO, DNB	Clinician, Chairperson	Male	No	Yes
Dr S Bhuvaneshwari	M.D	Clinical Pharmacologist Member - Secretary	Female	Yes	Yes
Dr Sudha Ramalingam	M.D	Epidemiologist Alt. Member - Secretary	Female	Yes	Yes
Dr Y S Sivan	Ph D	Member –Social Scientist	Male	Yes	Yes
Dr D Vijaya	Ph D	Member – Basic Scientist	Female	Yes	Yes

The approval is valid for one year.

We request you to intimate the date of initiation of the study to IHEC, PSG IMS&R and also, after completion of the project, please submit completion report to IHEC.

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This Ethics Committee is organized and operates according to Good Clinical Practice and Schedule Y requirements.

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Kindly note this approval is subject to ratification in the forthcoming full board review meeting of the IHEC.

Yours truly,

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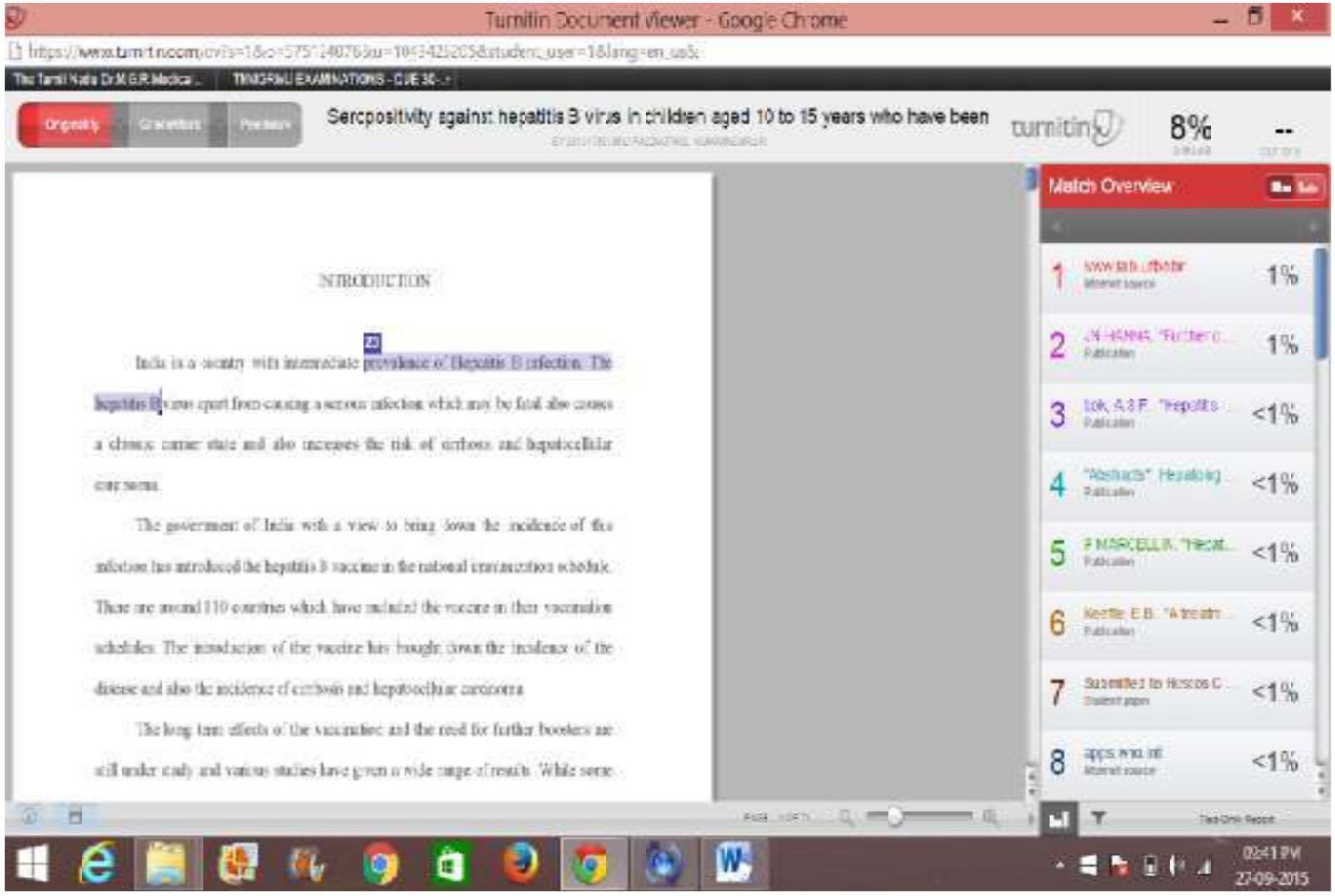
INTRODUCTION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INTRODUCTION
India is a country with intermediate prevalence of Hepatitis B infection. The hepatitis B virus apart from causing a serious infection which may be fatal also causes a chronic carrier state and also increases the risk of cirrhosis and hepatocellular carcinoma.
The government of India with a view to bring down the incidence of this infection has introduced the hepatitis B vaccine in the national immunization schedule. There are around 110 countries which have included the vaccine in their vaccination schedules. The introduction of the vaccine has brought down the incidence of the disease and also the incidence of cirrhosis and hepatocellular carcinoma.
The long term effects of the vaccinates and the need for further boosters are still under study and various studies have given a wide range of results. While some
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I dedicate this work with due respect and gratitude to all my teachers in the Department of Pediatrics, PSGIMS&R.

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PREVALENCE OF SEROPOSITIVITY AGAINST HEPATITIS B VIRUS IN CHILDREN AGED 10 TO 15 YEARS WHO HAVE BEEN IMMUNIZED AGAINST HEPATITIS B IN INFANCY

BACKGROUND:

Less is known regarding the duration and long term protection offered by hepatitis B vaccination and whether there is a need for booster doses of hepatitis B vaccine. This study determines the level of antibodies to hepatitis B surface antigen and analyses the prevalence of seropositivity. Though there are a number of studies from various countries, there are no Indian studies in this regard.

METHODS:

Children aged 10 to 15 years who have received three hepatitis B vaccination doses before one year of age and who have records of the same are recruited for the study. Records are verified for the immunization status and confirmed. After getting proper consent for the study from the parent and obtaining assent from the children, 3 ml of blood sample is taken and levels of anti HBs levels determined by chemiluminescence immunoassay technique.

RESULTS:

Of the 30 children aged 10 – 15 years who were vaccinated for hepatitis B before 1 year of age,who were included in the study only 9 children had anti HBs levels > 10mIU/mL.

CONCLUSIONS:

Only 30% of the children who were vaccinated with the three doses of hepatitis B before infancy had protective titers of anti HBs antibodies.

INTRODUCTION

India is a country with intermediate prevalence of Hepatitis B infection. The hepatitis B virus apart from causing a serious infection which may be fatal also causes a chronic carrier state and also increases the risk of cirrhosis and hepatocellular carcinoma.

The government of India with a view to bring down the incidence of this infection has introduced the hepatitis B vaccine in the national immunization schedule. There are around 110 countries which have included the vaccine in their vaccination schedules. The introduction of the vaccine has brought down the incidence of the disease and also the incidence of cirrhosis and hepatocellular carcinoma.

The long term effects of the vaccination and the need for further boosters are still under study and various studies have given a wide range of results. While some studies say the anamnestic response is good and rule out the need for boosters, some have come out that the anamnestic response may not be so good in all the vaccinees and stresses the need for pre vaccination testing and booster doses.

There are no studies from India in this regard. This study tries to throw light on the protectiveness of the hepatitis B vaccine in the Indian children aged 10 – 15 years who were vaccinated with the three primary doses of the vaccine before 1 year of age.This study may be a starting point for further studies in this regard from India.

AIM AND OBJECTIVES

PRIMARY AIM OF THE STUDY

To find out the prevalence of seropositivity against hepatitis B virus in children aged 10 to 15 years who have been immunized against hepatitis B in infancy.

SECONDARY AIMS OF STUDY

To look into other factors like age, sex on the anti HBs antibody levels.

NEED FOR THE STUDY

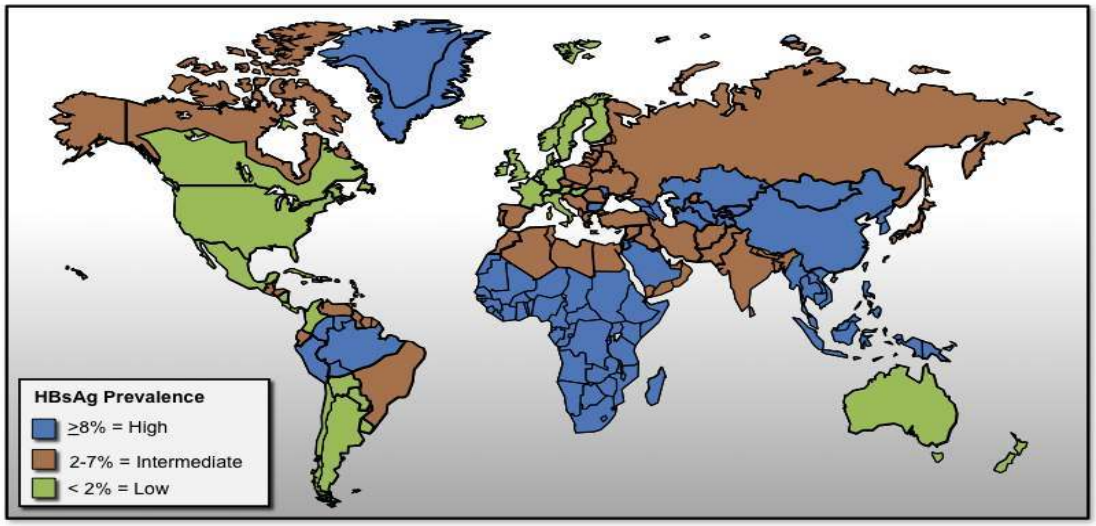
Less is known regarding the duration and long term protection offered by hepatitis B vaccination and whether there is a need for booster doses of hepatitis B vaccine. This study determines the level of antibodies to hepatis B surface antigen and analyses the prevalence of seropositivity. Though there are a number of studies in this regard, there are no Indian studies in this regard.

REVIEW OF LITERATURE

Hepatitis Bis an infection is due to hepatitis B virus and causes potential life threatening infection of liver. Apart from the acute infection, it can cause a chronic infection and increases the chances of cirrhosis, hepatocellular carcinoma and death in those who are chronically infected. It can also produce a chronic carrier state who transmit the virus continuously. Because of all these characteristics it poses a major global health issue.

The highest prevalence of this infection is found in east Asia and some parts of sub-saharan Africa. In these areas around 10% of the adults are infected chronically with this virus. High chronic infection rates is also found inAmazon and parts of eastern and central Europian countries. The Indian sub-continent and Middle East have around 5% of the population having chronic disease. Western Europe and northern America have less than 1% chronically affected individuals in the general population.

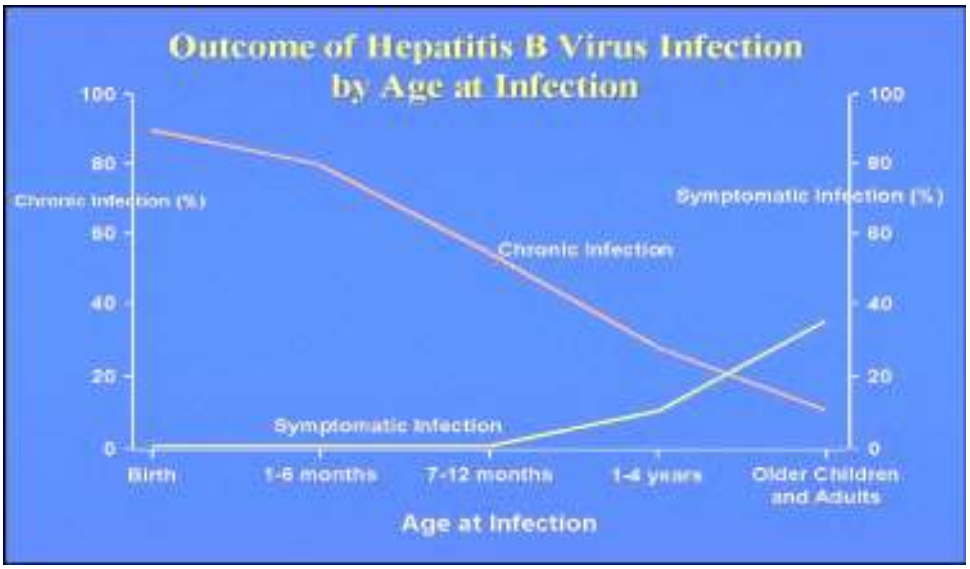
Figure 1 : Prevalence of hepatitis B infection



The virus is transmitted either by vertical transmission from HBsAg positive mother or is transmitted horizontally through cutaneous or mucous membrane exposure to infected body fluids like blood, saliva, vaginal, seminal and menstrual fluids. Hepatitis B spreads through sexual route in men having sex with men and men who have multiple sexual partners and contact with commercial sex workers. Infection can also occur through used syringes either in a health care setting or IV drug abusers. The infection can also spread during medical or surgical procedures or through objects contaminated with infected blood like tattooing, razors and similar objects.

The hepatitis B virus has the ability to survive out of the human body for upto 7 days. The virus is infectious even during this period. The incubation period of hepatitis B virus can vary from 30 to upto 180 days. This virus can be identified within 30 to 60 days after infection. Children less than 5 years who acquire the infection have a higher chance of developing a chronic infection than adults.

Figure 2 : Outcome of HBV infection by age at infection



A vaccine for hepatitis B is available since 1982. Recombinant vaccines became available in the mid-80s. The vaccines are very much effective in preventing the hepatitis B infection and also have substantially brought down the rates of complications like cirrhosis and hepatocellular carcinoma.

WHO recommends all countries to include hepatitis B vaccination in their immunization schedule. So far 110 countries have adopted the policy of vaccinating all children for hepatitis B. In India also the government has included hepatitis B vaccination in the national immunization schedule.

The vaccine has an excellent efficacy and has a very high seroconversion rates. The efficacy of the vaccine is also said to last long and hence as of now booster doses are not recommended. Various studies have been conducted and still others underway to determine the long term effectiveness of the vaccine and the need for booster doses.

Since there are no studies from India in this regard this study may be a starting point for further larger studies in evaluating the effectiveness and the protection offered by the hepatitis B vaccine among Indian population over a long period.

CHARACTERISTICS OF THE HEPATITIS B VIRUS

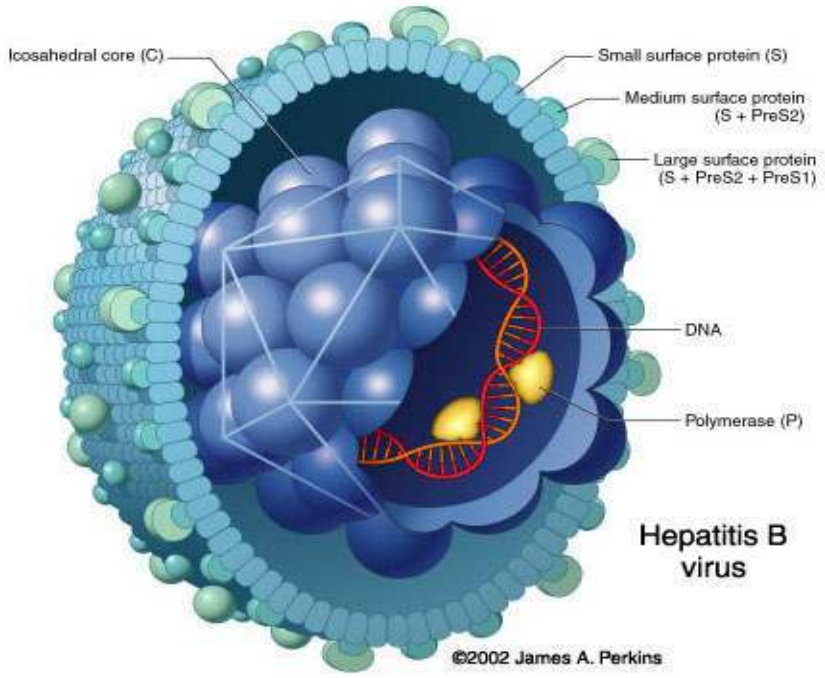


Figure 3: structure of the hepatitis B virus

Hepatitis b virus comes among DNA virus and belongs to the family hepadnavirus. The total size of the virus or is 42 nm in diameter also called the dane particle. It has an envelope composed of viral proteins and lipid components of the host and the core which is formed of the viral genome, nucleocapsid protein and the polymerase protein. The virus also gives rise to sub viral particles of size 22 nm containing envelope protein only without the genome of hepatitis B virus. These are noninfectious. The virus has a genome of relaxed and circular, DNA which is partially double stranded with approximately 32,000 base pairs[1].

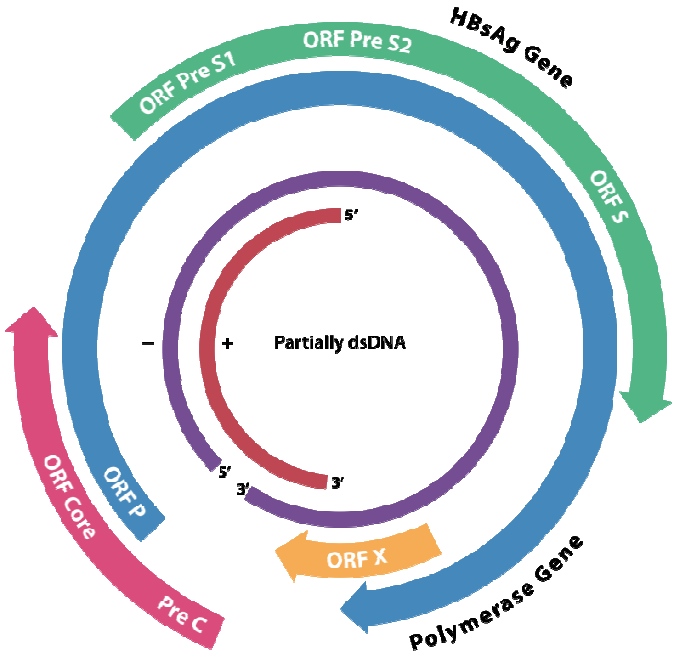


Figure 4 : genome organization of HBV

The DNA contains four open reading frames (ORFs) which are partially overlapping. These encode the core (pre core/core), envelope (pre S/S), X protein and polymerase. There are is a common stop codon in pre S/S ORF which divides the gene into three regions pre S1 encoding the large (L) envelope protein, pre S2 encoding the middle (M) envelope protein and pre S3 encoding the small (S) envelope protein and three phase start codons. All viral and sub viral particles contain the M and S envelope protein in contrast, L envelope protein is found mostly in complete virions[1].

There are two in phase start codons in the pre core/core ORF. Translation from the first pre core start codon gives rise to a pre core polypeptide that is then modified post translationally into HBeAg which is a soluble protein. The second pre core start codon produces HBcAg, the core protein. There is overlapping of the polymerase

ORF with core,envelope and X ORFs. The polymerase protein comprises of a primer, spacer, RNAase H domain and a reverse transcriptase/DNA polymerase.

The X protein is an effective transcriptional trans-activator that activates a lot of promoters which includes HBV and cellular oncogenes. And this X protein is not essential for replication of virus. It has been implicated in hepato-carcinogenesis. There are two major transcripts that are made, a 3.5 kb and a 2.1 kb transcript. There is a heterogeneous 5' end in the 3.5 kb RNA. pre core mRNA is longer & is translated to a pre core polypeptide and this is processed at both the N and C terminal ends to HbeAg, a smaller protein. Within the pre core region, the pre genomic RNA is initiated and serves as a replicative intermediate. It is reverse transcribed into HBV DNA. It also functions as a mRNA for translation into the polymerase proteins and nucleocapsid.Within the pre S1 region, the 2.1 kb RNA is initiated and is translated into middle and small S proteins. Apart from this, there are at least two minor transcripts. The 2.4 kb RNA that is translated into the large S protein and a smaller RNA that is translated into the X protein.

GENOTYPES OF HEPATITIS B VIRUS

Depending on an inter group divergence in the complete nucleotide sequence of eight percent or more, the hepatitis B virus is categorised into 10 genotypes named A to J. these genotypes have been associated with HCC development and a interferon therapy response.

REPRESENTATION OF THE REPLICATION CYCLE OF HBV

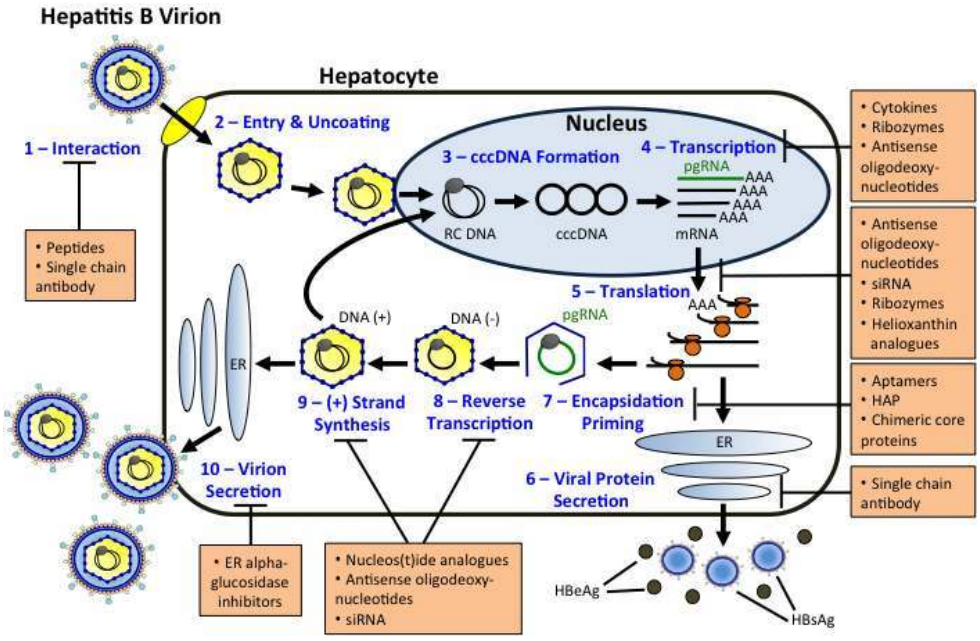


Figure5 : Demonstration of the events in replication of HBV

Hepatitis B virus attaches initially to the hepatocyte membrane. The receptor has been identified to be a bile salt transporter, sodium taurocholate co transporting polypeptide (NTCP), which is also the receptor for hepatitis D virus. This receptor binds to the preS1 region of envelope of hepatitis B virus. There is uncoating of the

virion into the hepatocyte cytoplasm and the genome of the virus enters the nucleus of hepatocyte. Synthesis of the plus strand HBV DNA is completed inside the nucleus of hepatocyte and the viral genome is then converted into a covalently closed circular DNA (ccc DNA).

Hepatitis b viral genome, undergoes replication by a process of reverse transcription through the pre genomic RNA, a RNA intermediate. There is encapsidation of the nucleocapsid, polymerase proteins andpre genomic RNA in the virus core particle and inside which the reverse transcription takes place. Pre genome encapsidationsequence is located in pre core and proximal core region which regulates encapsidation. The only RNA transcript that is encapsidated is the pre genomic RNA.

There is a new minus strand that is produced and is then followed by production of new plus strand DNA. The nucleocapsidsalong with the partially double stranded HBV DNA reenter the nucleus of the hepatocyte and result in production more of covalently closed circular DNA or are secreted as complete virions after being coated with envelope proteins. This covalently closed circular DNA that is produced has a long half-life. Owing to this fact there is thus a difficulty in achieving complete clearance during therapy with antivirals.

PATHOGENESIS OF INFECTION

Immune mediated mechanisms are implicated in the pathogenesis of liver disease due to hepatitis B virus. Direct cytotoxic injury to the liver is also reported. Liver disease related to HBV may be due to both hepatocyte lysis which is cytotoxic T cell mediated and also due to non cytolytic T cell mediated immune response.

The observations which are consistent with this hypothesis are

- Immune clearance associated events such HBeAg seroconversion that may be spontaneous or interferon induced are associated with liver disease exacerbation which is evident by an elevated ALT levels in the serum [2].

- Patients having chronic hepatitis B with HBeAg clearance have a vigorous cytotoxic T cell response to the antigens of HBV than those who are positive for HBeAg. However the T cell response of those in immune tolerance phase is not weaker than that of those in immune active phase in HBeAg positive patients. This is necessarily a difference in the inflammatory response and not of T cell response [3].

- The immune mediated lysis of hepatocytes that are infected is responsible for fulminant hepatitis. This is the reason why there is no evidence of replication of HBV in patients having fulminant hepatitis B.

The T cell response and antibody response to HBV help to control infection apart from promoting hepatic injury. In a study where patients were followed for 23 years after serological and clinical recovery, the cytotoxic T cells expressed activation markers which were suggestive of a recent contact with HBV antigens. This is suggestive of the fact that HBV cannot be completely eradicated and the traces of the virus present can enable the immune response to be maintained for years after recovering from the acute illness [4,5].

In another study involving five patients who were picked up during the incubation period of the acute illness, the first thing that was identified was NK cell activation. This was followed by T cell response specific to HBV. CD8+ and CD4+ cells specific to HBV present during the incubation period is suggestive of the fact that these cells have an important role in infection control and also in the events that leads to liver damage [6]. The peak reduction in HBVDNA levels usually occur before the peak increase in ALT levels which is suggestive of the fact that viral control is both by cytolytic and non cytolytic mechanisms.

DIRECT CYTOTOXIC LIVER INJURY

HBV is not among the cytotopic viruses. There may be no major concordance between the actual severity of the liver disease and viral load present in the patients having chronic hepatitis. For instance in HBV infection that is acquired at the time of birth (perinatal period), the load of HBVDNA is very much increased but inspite of it, the serum ALT levels are normal. However in rare conditions such as

fibrosingcholestatic hepatitis, rather an unusual form of liver disease seen in some patients with post liver transplantation recurrent hepatitis, there can be direct liver injury when there is a high viral load[7].

ROLE OF VIRAL VARIANTS

In patients with chronic hepatitis B infection it has been observed that there occurs a mutation of various regions of HBV. Some of these mutations involving the pre core stop codon have been responsible for causing severe liver disease. These mutations have also been noticed in asymptomatic carriers. Hence mutations alone are not pathogenic but they have a role in altering the replication of HBV or expression of immunogenic epitopes and thus may modulate potentially the severity of liver disease. Some variants though may not make HBeAg, can continue to replicate the virus and transmit the infection [8, 9]. The patients progressing to chronic HBV infection, have a limited and weak response to epitopes of HBV [10].

CLINICAL MANIFESTATION AND NATURAL HISTORY

The clinical manifestations of hepatitis B infection has a wide spectrum both in acute and chronic diseases. In case of the acute phase, the spectrum varies between anicteric to icteric hepatitis, in a few cases there occurs a fulminant hepatitis. In case of the chronic phase of infection, the spectrum varies from asymptomatic carriers to chronic cirrhosis and even carcinoma of the liver. Extra hepatic manifestations can be present in both types of the disease.

ACUTE HEPATITIS

There is a wide spectrum of the clinicalpresentations of acute hepatitis. Approximately 70 percent of them are either anicteric or have a subclinical course of hepatitis. The 30 percent of them present with icterus. When there is co-existing infection with other hepatitis viruses, the disease may be more severe [11].

On an average 0.1 to 0.5 percent may develop fulminant hepatitis as a result of immune mediated lysis of infected hepatocytes.The reason for some patients developing fulminant hepatitis not clearly known [12]. A study evaluating the risk factors for fulminant hepatitis concluded that patients who had lost weight in the past six months, alcoholics or who use methamphetamine were associated with greater risk of developing fulminant hepatitis. Also it was noted that all the isolated viruses were of genotype D[13]. Hence it is not sure whether it is the environmental factors or the viral factors which lead to fulminant hepatitis.

MODE OF TRANSMISSION

The mode of transmission of hepatitis B virus varies according to the region and prevalence rates. In areas with high prevalence rates such as South east Asia and China there is higher rates of perinatal transmission and occasional horizontal transmission early in life. In the United states, Canada, and western Europe, sexual contact and percutaneous transmission is common.

COURSE OF ILLNESS

After being infected, there is an incubation period which may last from one to up to four months. Then there is a prodromal phase when there may be a serum sickness like syndrome following which constitutional symptoms may occur which may include nausea, anorexia, jaundice and pain in the right hypochondrium. These symptoms gradually disappear one after other over a period of 30- 90 days but a few of them may have prolonged symptoms even after the aminotransferase levels return to normal.

INVESTIGATIONS

In the acute phase there is an elevated alaninetransaminase (ALT) and aspartate transaminase (AST) levels. The values may be as high as 1000 to 2000 IU/L and ALT is greater than AST. In case of anicteric hepatitis the bilirubin levels may be within normal limits.

The best indicator of prognosis is however the prothrombin time. After recovery the amino transferase levels normalize in one to four months.

Persistence of elevated amminotransferase levels for greater than six months is an indication of progress to chronic hepatitis.

OUTCOME

It is thought that in patients after recovery from acute illness, there is complete clearance of virus by antibodies and cytotoxic T lymphocytes but traces of the hepatitis B virus are often detectable by PCR in the blood for even many years after complete clinical recovery even though serum antibodies and cytotoxic T cells specific to HBV are present [14]. There are various studies which followed up the patients after recovery.

In a study it was found that activation markers were expressed in cytotoxic T cells 23 years after recovery indicating recent antigen contact. In another study 13 of 14 healthy liver transplant donors had HBV DNA detected in the liver who tested positive for anti HBc and anti-HBs antibodies [15]. In another series involving nine patients after complete recovery, histological changes (which include mild inflammation and fibrosis) were present for around 10 years [16].

These studies suggest that, the latent infection persisting can maintain the T cell response for years and hence keeping the virus under control. Hence a complete recovery after acute HBV infection is rare . [14]. There are smaller studies which suggest that there may be liver damage following latent infections. Immunosuppression in these patients can result in re activation of hepatitis B virus.

The age at the time of infection determines the rate of progression to chronic hepatitis B. For perinatally acquired infection there is a 90 percent chance of progression to chronic disease, 20 to 50 percent chance if the infection occurs between one and five years of age and less than 5 percent chance if the infection is acquired in an adult.

TREATMENT

Supportive care is the main treatment in acute hepatitis B infection. The indications for hospitalization are mainly coagulopathy, deep jaundice or encephalopathy. The decision to hospitalize depends on the individual and older patients, patients with co morbidities, those who cannot take orally and those with poor socio economic support can also be considered for admission.

Treatment should also be aimed at preventing infection in exposed contacts. As far as the role of antivirals are considered, not all patients with acute hepatitis B require treatment with nucleoside or nucleotide since there is less than 1 percent chance of developing fulminant hepatitis and also there is less chance of progression to chronic hepatitis B in adults with normal immunity.

A placebo controlled trial which included 71 acute hepatitis patients (with a subset of 47 with severe hepatitis B) who were randomized into 31 receiving lamivudine and 40 receiving placebo for three months concluded that there was no clinical or biochemical benefits to therapy with lamivudine. Between the two groups there was no difference in HBsAg loss noted at 12 months. The limitations of this

study were that the duration of illness was not mentioned and only 3 out of 47 had encephalopathy [17].

Antivirals are indicated in patients with severe (eg. Coagulopathy INR>1.5) or a protracted course (marked jaundice bilirubin >10mg/dL or persistence of symptoms for greater than four weeks after presentation).

Patients with fulminant hepatitis are also treated with antivirals to the reduce the chances of reinfection after liver transplant and also in immunocompromised who have additional infection with hepatitis C or D virus, those having a preexisting hepatic disease or elderly patients are also treated with antivirals.

Interferon has an increased risk of hepatic necroinflamation and should be avoided. Monotherapy can be given with entecavir, tenofovir, lamivudine, telbivudine or adefovir. The total duration of monotherapy treatment should be short and treatment should be stopped after confirming the clearance of HBsAg.

CHRONIC HEPATITIS

Only a small patients with chronic hepatitis would give a history of acute hepatitis in the past. In the areas where there is intermediate or low prevalances, history of acute hepatitis could be elicited in 30 - 40 percent of chronic HBV infection patients. In areas of high prevalence, most of them present are symptomatic whereas others may have nonspecific symptoms such as fatigue.

These asymptomatic patients may present with decompensated cirrhosis or with extra hepatic manifestations. There may be exacerbations in some patients which may at times present as hepatic failure or may mimic acute hepatitis or may even be asymptomatic.

Patient with chronic hepatitis may have a normal physical examination or can have stigmata of chronic liver disease. Icterus, peripheral edema, ascites, encephalopathy and splenomegaly may be noted in case of decompensated liver disease.

INVESTIGATIONS

Investigations may have a mild to moderate elevation of transaminasesor may be normal. The ALT concentrations may go up to 50 times the normal values in case of acute exacerbations. The alphafetoprotein (AFP) levels may reach upto 1000 ng/mL [18].Features of hypersplenism such as decreased platelet counts and WBC and features of decreased liver function such as hyperbilirubinemia, hypoalbuminemia and prolonged PT should raise a suspicion of cirrhosis.

EXTRAHEPATIC MANIFESTATIONS

Circulating immune complexes are thought to be responsible for the extrahepatic manifestations occurring in 10 to 20 percent of chronic HBV patients. The disease may start as a serum sickness like illness which is clinically presents as fever with rash, arthritis or arthralgia. These symptoms usually regress when jaundice occurs.

The main extra hepatic complications of chronic HBV are as follows

- **Glomerular disease -** HBV most commonly induces membranous nephropathy and to a lesser extent membranoproliferativetype of glomerulonephritis. The HBV related glomerular disease most commonly occurs in children. These children usually present with nephrotic range proteinuria and out of these children almost 30 to 50 percent undergo spontaneous remission that is usually associated with seroconversion of HBeAg to anti HBe. In adults there may be a progression to renal failure. The benefit of antivirals in glomerular disease is not clear.
- **Polyarterirtisnodosa –** the clinical manifestations are no different from patients having HBV negative polyarteritis. HBV related polyarteritis patients can have benefit by antiviral therapy.

- **Aplastic anemia –** aplastic anemia has been seen in association with hepatitis B infection but most of them may not be due to HBV.

PHASES OF CHRONIC HBV INFECTION

The virus replication and host immune response decide the natural course of chronic hepatitis B infection. Other elements which are likely to have a role in the worsening of the disease are gender, alcohol consumption and co infection with other hepatitis viruses. The severity of liver disease when the HBV replication is arrested decides the outcome of chronic hepatitis B infection.

There are two phases in chronic HBV infection. An early replicative phase associated with active liver disease and a late phase with reduced replication and remission of the disease. There is an additional phase of immune tolerance in cases of HBV infection acquired perinatally where there is viral replication but no active liver disease. There may be a re activation of HBV replication after a period of quiescence in some patients.

REPLICATION PHASE

- **Immune tolerance phase**

In perinatally acquired HBV infection, there is a phase of immune tolerance in which there HBV replication takes place in high levels characterized by high HBV DNA levels in the serum of the patient and presence of HBeAg but there is no evidence to suggest that there is an active liver disease. There are no symptoms ant investigations show normal ALT concentrations and with no major changes in liver biopsy.

There are two studies which concluded that stage 0 fibrosis was found in 30 to 50 percent of patients in the replicative phase. The others in this phase had grade I fibrosis. One of the two studies showed unchanged fibrosis scores after five years among patients in immune tolerance phase. Immune tolerance to HBV is responsible for the absence of liver disease although there are high hep B viral replication levels [19, 20].

Maternal HBe Ag transfer transplacentally induces specific unresponsiveness of T lymphocytes to HBcAg and HBeAntigen and as a result of which in infected hepatocytes there is ineffective cytotoxic T cell lysis. This has been demonstrated by experiments in mice. The poor response to therapy with interferon in Asian patients with positive HBe Antigen and normal ALT levels can be attributed to immune tolerance to hepatitis B virus.

But there are studies which have found no difference in T lymphocyte response to HBV during the replicative phase which challenges the concept of immune tolerance [21]. The rate of spontaneous clearance of HBeAg is low during the immune tolerance phase and may last for 10 to 30 years.

Studies involving Chinese children have found that 90 percent of children below five years of age have HBeAg and in children below 20 years HBeAg is found in upto 80 percent. Spontaneous HBeAg clearance occurs only in 2 percent in the first three years and 20 years after infection it is only 15 percent. This low rate of clearance

of the virus in the early adulthood and in adolescence is responsible for the high rate of vertical transmission in Asian countries [22].

- **Immune clearance phase**

Transition to this phase from immune tolerance phase usually occurs in 20-30 years of age in cases with perinatallytransmitted HBV infection.

In this phase, rate of spontaneous clearance of HBeAg increases to 10 to 20 percent every year. In a study involving 1536 Alaskan people a 10 year seroconversion rate of 70 percent was observed in adults who acquired HBV during adulthood [23]. Several studies from Asia have reported an earlier seroconversionin infection with HBV genotype B rather than in those people who are infected with genotype C. This seroconversion may be but not always have a concominant biochemical exacerbation.

Usually there is an abrupt elevation of serum ALT levels which is due to lysis of infected hepatocytes by immune mediated mechanisms. This elevation is usually begins with an elevation in serum HBV DNA and HBcAg shift from nuclear to cytoplasmic sites in the hepatocytes. These changes suggest that an increased viral load or change in viral antigen presentation triggers the immune clearance but the mechanism is not clear.

The exacerbations are usually asymptomatic and may be noticed during regular follow up. But sometimes the exacerbations may have symptoms of acute hepatitis B infection and may be a reason for incorrect diagnosis if a history of previous hepatitis

infection is not available. IgMantiHBc titer may be elevated which leads to misdiagnosis as acute hepatitis B.

Elevation of serum alphafetoprotein levels may be a concern for the diagnosis of hepatocellular carcinoma.

The exacerbations have a male predominance and the reason for this gender difference is not known but this higher exacerbation rates in men may be a reason for higher incidence of cirrhosis related to HBV and hepatocellular carcinoma in men. The exacerbations may lead to hepatic decompensation and rarely to hepatic failure and death in a few patients [24]. Decompensation in HBeAg positive patients not having cirrhosis can be predicted by serum Hepatitis B viral DNA levels of greater than 1.55×10^9 copies/mL during the onset of flare.

Interferon is not indicated in the treatment of acute exacerbation as additional exacerbation. Patients in exacerbation should be treated with nucleoside or nucleotide analogues and should be referred to specialized or tertiary care hospitals with liver transplantation facilities.

There is a phenomenon called adoptive immune clearance which occurs in some patients where during exacerbations there in no HBeAntigenseroconversion and clearance from the serum of hepatitis B viral DNA. In such patients, there may be recurrent exacerbations and disappearance of the DNA from the serum intermittently which may or may not be associated with a transient HBeAg loss.These repeated

episodes may result in increased risk of hepatic cirrhosis and hepatocellular carcinoma.

A study involving 151 patients having Hepatitis B e antigen positive chronic hepatitis B with genotype C concluded that seroconversion of HBeAg was more in patients with HBV infection acquired after the perinatal period and also in those with HBV DNA levels ≤ 7 log copies/mL.

The HBeAntigen prevalence in patients with chronic hepatitis B infection is 10 to percent in non Asian adults whereas in chinese adults it is around 30 to 50 percent. But the spontaneous HBeAg clearance rate is about 10 to 20 percent every year in both these ethnic groups.

NON REPLICATION PHASE OR INACTIVE CARRIER STATE

The patients in this state are HBeAg negative but are positive for anti HBe. HBV DNA may be undetectable in the serum in these patients even by polymerase chain reaction assays and the serum ALT levels are normal indicating liver disease remission and liver biopsy may show resolution of necroinflamation. There are various studies which have looked into the carrier states.

In one of the studies, 40 percent of the carriers had hepatitis B DNA levels of 10^4 copies/mL or more [25].

A meta-analysis found that in patients with HBV DNA levels ≤ 20,000 IU/mL and normal ALT, the incidence of liver disease was rare. Still patients with normal ALT can have histologically significant inflammation or fibrosis [26].

In another study where patients with HBV DNA levels > 4 log copies/mL were alone biopsied, age was one of the predictors of significant histologic findings [27].

In a third study, it was reported that in 21 percent of patients with negative HBeAg, persistently normal alanine aminotransferase levels and hepatitis B DNA levls< 5 log cop/mL there was histopathologically active liver disease. But in this study not all patients with HBeAg negativity, persistently normal alanine aminotransferase levels and Hep B DNA <5 log cop/mL were biopsied and hence could not be taken as a representation of the entire group [28].

But all of these datas conclude that, significant hepatic disease may be present in patients with chronic HBV with negative HBeAg but is a rare entity in patients withpersistently normal alanine aminotransferase levels which means at least three normal ALT levels in a 12 month period and hepatitis B DNA < 4 log cop/mL.

Since the course of chronic HBV infection is fluctuating patients should be classified as inactive carriers only if there are

1. Three normal ALT levels over a 12 month period and
2. Two to three < 4 log copies/mL HBV DNA levels over a 12 month period.

HEPATITIS B ENVELOPE ANTIGEN NEGATIVE CHRONIC HEPATITIS

In some patients in spite of remaining HBeAg negative may have moderate HBV replication and elevated alanine aminotransferase levels and chronic inflammation in liver biopsy indicating an active liver disease. They have a wild type of virus or a variant of HBV which is unable to produce HBeAg because of a pre core or core promoter variations. These patients fall under the category of HBeAg negative chronic hepatitis [29]. These patients usually have fluctuations in HBV DNA and alanine aminotransferase levels and are generally older and with advanced liver disease.

In a study involving 217 asymptomatic HBeAntigen negative, anti HBelevels positive chronic HBV patients having normal baseline ALT levels, the frequency of flares were estimated for a median follow up period of six years. In 43 patients there were spontaneous ALT flares. The cumulative probability of a flare was 47 percent at 10 years and 11 percent in 5 years. Significant association of flare was found in age more than 30, male gender and the presence of pre core mutation [30].

Follow up once in three months captured around 90 percent of flares. Cirrhosis and hepatocellular carcinoma risk were negligible in those who had a sustained remission and were much significantly high in those with elevated ALT even after HBeAg seroconversion [31].

RESOLUTION OF CHRONIC HEP. B VIRUS INFECTION

HBsAg becomes negative in some patients with chronic hep B. 0.5 percent to 2 percent is the annual rate of clearance of HBsAg reported in western patients and is supposed to be much lower in Asian countries. A study done in Taiwan gives a cumulative probability of HBsAgseroclearance as 8 percent after 10 years but showed an increase to 25 percent after 20 years and 45 percent after 25 years [32].

Another study where 3087 patients, most of them HBeAgseronegative were followed up, the annual clearance of HBsAg was 2.26 percent. A decrease in HBV DNA levels preceded this clearance. The HBsAg cumulative clearance was 26 percent at 5 years and 51 percent at 8 years [33].

In most of these studies the prognosis was good in patients who dint have cirrhosis and cleared HBsAg. Patients with concurrent HepatititsC or HDV infection had a higher rate of complications [34, 35].

PROGNOSIS AND SEQUALAE OF CHRONIC HEPATIS B

Chronic hepatitis B associated sequale may vary considerably. The sequelae may be an inactive carrier state, cirrhosis, liver decompensation, hepatocellular carcinoma, extra-hepatic manifestations and also death. Prognosis may vary according each individuals.

In two studies where the HBsAg positive blood donors were followed up for many years is was found that subjects with HBsAg positivity had no increased

incidence of clinically significant liver disease or the complications or death due to hepatic cause compared to HBsAg negative donors [36].

The prognosis is bad in patients living in endemic areas and those who have chronichep B infection.

The estimated rate of progression to the various complications over five year are [37]

12 to 20 percent for Chronic hepatitis to cirrhosis

20 to 23 percent for Compensated cirrhosis to hepatic decompensation

6 to 15 percent for Compensated cirrhosis to HCC

The cumulative survival rates for these stages of chronic disease are

85 percent in five years for Compensated cirrhosis

55 to 70 percent in one year and 14 to 35 percent in five years for Decompensated cirrhosis.

The lifetime risk of death due to liver disease is estimated to be around 40 to 50 percent in men and in women 15 percent in chinese patients with chronic hep B infection.

The risk progression is more in the following settings

In patients who had an immune clearance phase

In patients with delayed HBeAgseroconversion

Reactivation of the disease after HBeAgseroconversion.

The prognosis and survival rates have improved over the last ten years after the introduction of nucleotide and nucleoside analogues.

FACTORS PREDICTING DISEASE PROGRESSION

There are both virologic and non virologic factors which have an influence on survival and disease progression among patients with chronic Hepatitis B infection.

The HBeAg status of the individual, HBV DNA levels, HBsAglevls, genotype of HBV and certain HBV variants are the viral factors which decide disease progress.

Patients who have a prolonged replication phase are HBeAg positive and have a worse prognosis mainly because of the resultant chronic cirrhosis and hepatocellular carcinoma [38].

Patients with high Hepatitis B DNA levels have an high chances of developing cirrhosis, hepatocellular carcinoma, and mortality related to liver disease and hence a poor prognosis[39].

In chronic HBV patients with a negative HBeAntigen and a low viral load, HBsAg levels >1000 IU/mL is associated with an increased risk of hepatocellular carcinoma and liver related deaths [40].

In those with high viral load (≥2000 IU/mL), HBV DNA level is the most important predictor and in patients with viral load <2000 IU/mL, HBsAg levels is the important predicting factor.

Factors not associated with HBV which affect disease progression include

Host factors - gender, age, diabetes

Environment factors - alcohol, smoking, carcinogens

Coinfection with other hepatitis viruses (eg. HCV, HDV) and HIV virus

Markers of HBV in serum are found two to four fold higher in alcoholics compared to nonalcoholic controls. But there not much evidence suggesting those whoc consume alcohol have an increased probability of HBV infection. The detection of HBV DNA in alcoholics with liver disorder suggests that infection may be responsible for the liver disease in these patients. Accelerated liver injury and increased risk for cirrhosis and hepatocellular cancer have been reported in alcoholics [41].

HCV is associated with 10-15 percent of those with chronic hepatitis B infection. These patients have reduced HBV DNA levels and also increased rate of HBsAgseroconversion. In many patients with such co infection there may be detectable serum HCV RNA but low or very low serum HBV DNA. The duration of antigenemia, severity of liver incidence and chances of hepatocellular carcinoma are less in patients with combined HCV with HBV infection in comparison with those with isolated HBV infection. But increased risk of severe hepatitis and hepatic failure have been reported with acute HCV and HBV coinfection or an acute HCV infection in a patient with preexisting chronic HBV infection[42, 43].

Co infection with hepatitis D virus tends to be more severe compared to infection with hepatitis B virus alone. The HDV requires the presence of HBV for assembly of virion and secretion even though it can replicate autonomously. So HDV infection always occurs with HBV infection. Co infection with HDV infection leads to suppressed HBV replication due to interference mechanisms and the chances of development of a severe disease and fulminant hepatic failure is more compared to infection with hep B virus alone [44].

In co infection with hepatitis B, C and D infection, the interaction of viral replication is still unclear.There are some studies which show HDV to dominate and still others which show HCV to dominate in triple infections [45].

DIAGNOSIS OF HEPATITIS B

The diagnosis of hepatitis B viral infection can be made by detecting either hepatitis B antigens or their antibodies and by direct estimation of viral DNA which can be done by PCR assays.

Patients having active hepatitis or chronic liver diseases are evaluated for the presence of hepatitis B infection.

Screening for hepatitis B viral infection is done in the following conditions irrespective of their vaccination status [46]

- ✓ People born in countries where HBV prevalence is ≥2 percent
- ✓ Pregnant women during initial prenatal assessment.
- ✓ Those to be started on immunosuppressive therapy
- ✓ Persons infected with HIV or HCV
- ✓ Injection drug users
- ✓ Men having sex with men
- ✓ Persons with history of sexual diseases or those with multiple partners
- ✓ Patients who are on hemodialysis
- ✓ Persons having home or sexual contact with persons having HBV
- ✓ Inmates of correctional facilities

SEROLOGICAL MARKERS OF HEPATITIS B

Hepatitis B surface antigen HBsAgand antibody anti HBs

The serological hallmark of infection with hepatitis B is the appearance of hepatitis B surface antigen. It is detected mainly by radio or enzyme immune assays. It appears one to ten weeks after exposure and the appearance occur seven before the onset of symptoms of hepatitis or increase in ALT levels.

Incase of people who recover from the infection, HBsAg levels reach to under detectable levels in a period of around 4 to 6 months. The presence of HBs Antigen for more than six months is suggestive of chronic infection which may occur in around 1% of adult immune competent patients [47].

The disappearance from the blood of HBs Antigen is followed by the appearance of antibodies to these surface antigens (anti HBs). In majority of the patients anti HBs antibody persists throughout the life time and confers lifelong immunity. In some patients there is a window period where both HBs Antigens and anti HBs antibodies both are undetectable. In around 24 % of patients both HBsAg and anti HBs may be found. These patients may be taken as carriers of hepatitis B virus.

Hepatitis B core antigen HBcAgand antibody anti HBc

The core antigen (HBcAg) of the hepatitis B virus is an intracellular antigen and is expressed in the hepatic cells and not in the serum. Anti HBc antibody can be

detected in serum throughout the course of infection. During the acute phase of this infection, the antibody is predominantly of IgM type.

The detection of IgM anti HBc antibody is generally taken as a marker of acute Hepatitis B viral infection and may be the only marker in case of the window period of HBsAg. But IgM type anti HBc antibody can remain in detectable levels for around two years after an acute HB viral infection and also in patients who are having acute flare up of chronic hepatitis B infection posing a diagnostic problem in diagnosing acute hepatitis B in areas that are endemic for hepatitis B [48].

Patients who have recovered from acute hepatitis B have IgG anti HBc and anti HBs positivity. IgG anti HBc antibody is present along with HBsAg in cases of chronic HB viral infection.

Isolated anti HBc

The presence of antibodies to HB core antigen without the presence of HBs Antigen and anti HBs antibodies have been found in healthy blood donors ranging from 0.4 to 1.7 percent in areas of low prevalence and 10 to 20 percent in endemic areas [49].

Isolated anti HBc detection can occur in three circumstances

1. Window period in HB viral infection where anti HBc antibody is of IgM type
2. Years after recovering from an acute episode of HB infection where anti HBs antibody is low and not detectable

3. Many years after chronic HB viral infection where HBs Antigen titer has fallen less than the cut off point for detection.

HB viral DNA can been found in the serum in 0 to 20 percent and in the liver in more than 70 percent of patients with anti HBc positivity alone.

The chance of transmission is more when liver from an isolated anti HBc positive donor is transplanted compared to other organ transplantations.

Repeat testing for anti HBc antibody, HBs Antigen, anti HBs antibody and anti HBe antibody should be done after a period of six months in patients with isolated anti HBc positivity.

In patients with positive IgG anti HBc and a recent evidence of HBV exposure, IgM anti HBc levels must be done. In individuals having chronic liver disease testing for HBV DNA should be done.

Hepatitis B e antigen and antibody

The HBe Antigen belongs to a secretory protein and is produced from the pre core protein. This antigen is a marker of HB virus replication and its infectivity. If this antigen is present, it is associated with high serum HB virus DNA levels and higher rates of being transmitted from carrier mother to babies and to health care workers from patients [50, 51].

Seroconversion from HBeAg to antiHBe in patients with acute infection occurs early before seroconversion of HBsAntigen. Though in patients who have chronic hepatitis B, it may take years or decades for this seroconversion to occur. These patients have high HB virus DNA levels and active liver disease. In case of perinatally

acquired HBV infections, HBeAg positivity may be associated with normal ALT titers and little liver inflammation [52].

HBeAg to antiHBe seroconversionis mostly accompanied with decrease in serum HB virus DNA and remission of liver disease, though few patients may proceed to have active liver disease even after HBe Antigen seroconversion. In these individuals there may be low levels of wild type hepatitis B virus or HB virus variants that prevent or decrease HBeAg Production.

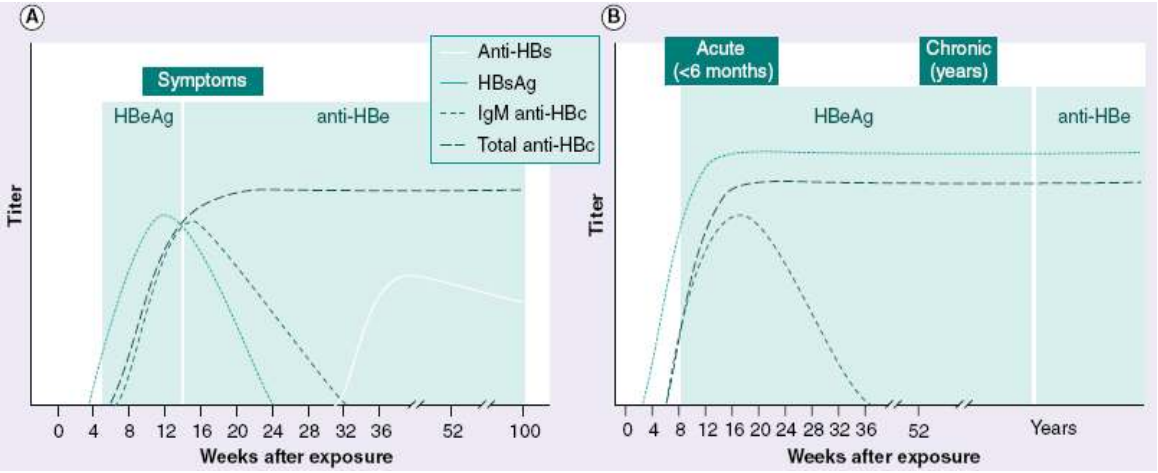


Figure 6 : Serological response to HBV infection

Serum HBVirus DNA assays

There are quantitative and qualitative tests available to detect HB virus DNA in the serum to find the replication of HBV. The sensitivity of these assays are dependent on the technique used. Real time PCR techniques are currently used for most HBV DNA assays.

The lowest limit of detection is about 10 to 20IU/mL and the linearity range is up to 8log(10)IU/mL.

Recovery from infection is usually associated with clearance of HBV DNA in serum. However in some patients HB viral DNA remains detectable in the serum for years especially if testing is done by PCR assays suggesting that the virus is persisting even after recovery. However it is controlled by the immune system. Findings similar to this have been noticed in patients who have chronic HB viral infection. HBeAg seroconversion, both that occur spontaneously or treatment induced is normally associated with the disappearance of serum HB viral DNA. But in case of PCR assays the HBV DNA may remain positive except in patients in whom there is HBsAg seroconversion.

Most patients on nucleoside or nucleotide analogue therapy who develop HBeAg seroconversionhave an undetectable HBV DNA levels. But despite this they may remain HBeAg positive for months or even years [53]. This may be due to the lack of effect of these drugs on the covalently closed circular HB viral DNA and also on viral transcription of RNA and on expression of viral protein.

Patients having HBe Antigen negative chronic hepatitis have detectable HB viral DNA levels though the levels are lesser than those in HBe Antigen positive patients. HBV DNA level of $>10^5$ copies/mL has been taken as the cutoff point in differentiating between HBeAg negative chronic hepatitis B and inactive carrier state.

DIAGNOSTIC ALGORITHMS FOR HEPATITIS B

Acute hepatitis

In the acute phase of the disease, HBsAg and IgM antiHBc are positive. HBeAg may be present in the initial phases of infection. Recovery from the acute phase is associated with the disappearance of HB viral DNA, seroconversion of HBe antigen to anti HBe antibody and then followed by seroconversion of HBsAg to anti HBs antibody.

In few rare instances there can be a window period where HBs antigen is negative but anti HBs antibody is not yet positive. In such situations that occur more commonly in patients having fulminant hepatitis B in whom viral clearance is more rapid, IgM anti HBc antibody is the marker of HB viral infection.

HBs antigen positivity can be seen in exacerbations or reactivation occurring in chronic hepatitis B and superinfection of a HB virus carrier with other hepatitis viruses or non-viral hepatitis in a HB viral carrier.

Past HB viral infection

The presence of past HBV infection is indicated by the presence of anti HBs antibody and IgG type anti HBc antibody.

Immunity post vaccinating has the presence of anti HBs antibody alone.

Chronic HBV infection

The presence of HBs antigen for greater than six months indicates chronic HB viral infection.

HBeAg and serum HBV DNA which are markers of viral replication should also be performed to decide whether the patient requires antiviral therapy. The patients having chronic HB viral infection should undergo regular monitoring as the HB viral DNA and ALT levels may vary and for monitoring the progression of liver disease [54].

Patients having normal ALT levels and low or undetectable levels of HB viral DNA are said to be in an inactive carrier state and have a very good prognosis.

In patients who are HBeAg negative and with elevated ALT levels, serum HBV DNA levels should be done to find out whether the liver disease is because of persistent HBV. Quantification of HBs antigen levels is used to differentiate inactive carriers from HBeAg negative patients.

Additional tests for hepatitis C virus and hepatitis D virus infection is also done to rule out superinfection.

Occult HBV infection

There is a group of patients said to have occult HBV infection characterized by the presence of detectable HB viral DNA by PCR and are negative for HBs antigen. These patients are classified into seropositive or seronegative HBV based upon the presence or absence of other HBV markers especially anti HBc [55]. Though the serum HB viral DNA levels in these patients are very low, patients having occult HB viral infection have an increased chance of chronic liver disease and hepatocellular carcinoma [55].

Table 1: Serological markers in different phases of HBV infection

HBsAg	HBeAg	IgM anti-HBc	IgG anti-HBc	Anti-HBs	Anti-HBe	HBV DNA	Interpretation
Acute HBV infection							
+	+	+				+++	Early phase
		+				+	Window phase
			+	+	+	±	Recovery phase
Chronic HBV infection							
+	+		+			+++	Replicative phase
+			+		+	±	Low, nonreplicative phase
+	±	+	+			+	Flare of chronic HBV
+			+		+	++	Precore/core promoter mutants
-	-		-			+	Occult HBV

Table 2: Interpretation of hepatitis B serological pannel

Tests	Results	Interpretation
HBsAg	Negative	Susceptible
anti-HBc	Negative	
anti-HBs	Negative	
HBsAg	Negative	Immune due to natural infection
anti-HBc	Positive	
anti-HBs	Positive	
HBsAg	Negative	Immune due to hepatitis B vaccination*
anti-HBc	Negative	
anti-HBs	Positive	
HBsAg	Positive	Acutely infected
anti-HBc	Positive	
IgM anti-HBc	Positive	
anti-HBs	Negative	
HBsAg	Positive	Chronically infected
anti-HBc	Positive	
IgM anti-HBc	Negative	
anti-HBs	Negative	
HBsAg	Negative	Four interpretations possible¶
anti-HBc	Positive	
anti-HBs	Negative	

The four possible interpretations in the last scenario are

- Recovery from acute HBV
- Distantly immune
- Susceptible to false positive anti HBc
- Chronically infected with undetectable HBsAg

The vaccines for hepatitis B that are available currently are extremely safe with an efficacy of > 90 percent and are active against all serotypes of HBV. Thus through a good vaccination coverage globally it is possible to eradicate hepatitis B infection.

Types of vaccines for hepatitis B

- **Plasma derived vaccines**

  The initial first generation hepatitis B vaccines were prepared from HBsAg carriers. The plasma was concentrated and purified to produce 22nm subviral particles. These vaccines had HBsAg alone and since they were plasma derived concerns regarding blood borne infections were high though this vaccine had an excellent efficacy [56].

- **Yeast derived recombinant Hepatitis B vaccine**

  These type of vaccines were introduced first in the mid-80s. They were produced by cloning the S gene of HB virus in yeast cells. These vaccines had non glycosylated HB virus small S protein and do not have the pre S region antigens. The initial concerns were regarding the thiomersal which was used as a preservative. Now thiomersal free yeast derived vaccines are readily available [57].

- **Mammalian cell derived Hepatitis B vaccine**

Three mammalian cell derived hepatitis B vaccine have been developed. One of these contain the pre S2 antigen in addition to the S antigen while the other tho vaccines in addition to the S antigen contain both pre S1 and pre S2 antigen.

These vaccines have been found to be more effective than the yeast derived vaccines. They are also equally effective in a two dose regimen compared to the conventional three dose regimen. Though these vaccines are highly efficacious they are not readily available and the cost is also high [58].

- **Vaccines under trials**

A novel hepatitis B vaccine which may be administered intranasally has been developed. It has the HB surface antigen and HB core antigen which is given as a nasal spray as a 5 dose schedule of 0, 7, 15, 30 and 60 days [59].

Another hepatitis B vaccine (HEPLISAV) which contains an adjuvant immunostimulatory phosphorothioate oligodeoxyribonucleotide (HBVISS) in addition to the hepatitis B surface antigen. This vaccine is supposed to give a higher rate of seroprotection in a two dose regimen [60].

Hepatitis B vaccine is available commercially in combination with Hepatitis A vaccine and also in combination with tetanus, pertussis, diptheria and hemophilus influenza type B vaccine.

CANDIDATES FOR VACCINATION

All neonates irrespective of the HBaAg status of the mother and all high risk groups who are not vaccinated and have not acquired the disease are candidates who should be vaccination.

Infants born to HBs antigen positive mothers

The most important step towards eradication of HB infection is the vaccination of all infants born to mothers who are HBs antigen positive. The regimen consists of both active as well as passive immunization at the same time. Both hepatitis B immunoglobulin and Hepatitis B vaccine are administered simultaneously at different sites within 12 hours after delivery. This combined regimen has an efficacy of 95 percent compared to an efficacy of around 70 to 75 percent for the administration of vaccine alone [61].

All new born

For the global eradication of hepatitis B viral infection it is strongly recommended that all neonates irrespective of the HBs antigen status of the mother are advised to receive the complete three doses of hepatitis B vaccine. The implementation of the universal vaccination for hepatitis B in many countries has led to the decrease in the hepatitis B infection rates and HBV related cirrhosis and hepatocellular carcinoma [62]. Universal vaccination can prevent both vertical and horizontal transmission of HB viral infection and also the sequelae of chronic HB infection. But in low endemic areas the benefits may not be evident until two to three

decades after as the infection in these countries is usually among adolescents and young adults where the transmission is through percutaneous or sexual route.

Premature infants

The hepatitis B vaccine is found to be less immunogenic in pre-term infants and in those infants with a birth weight <2 kg. Pre-term infants who are medically stable however respond to hepatitis B vaccine by 30 days of age irrespective of their gestational age and weight at birth. These infants are given the first dose at 1 month of age. The subsequent doses are given at a gap of one and six months [63].

Catch up vaccination

This refers to the vaccination of all children and all adolescents <19 years those who have not yet been earlier immunized. They should receive the three dose HBV vaccine series before they become adolescents when they are at a greater risk of HB infection through intravenous drug use and sexual route [64]. Vaccine coverage in the catch up immunization program varies widely from around 60 percent in US to around 51 to 67 in Asian and Pacific Islander children.

Other high risk groups

Some countries target the high risk group for vaccination. This approach did not bring any difference in the incidence of hepatitis B as many of the high risk persons were never identified. Nevertheless every possible attempt to vaccinate these individuals should be done [65]. The high risk groups include,

- Persons with multiple sex partners and males who are homosexual or bisexual.

- Contacts of patients having hepatitis B at home.
- Intravenous drug users
- Healthcare workers
- Patients who are on hemodialysis and those requiring repeated blood transfusions
- Patients with chronic liver disease
- Unvaccinated persons traveling to HB infection endemic areas
- Patients with diabetes

PRE VACCINATION SCREENING

The role of pre vaccination is to identify persons who are already infected and those who do not require vaccination.

Pre vaccination testing is unnecessary and not cost effective in non-endemic areas. But in case of endemic areas, the pre vaccination screening in addition to finding patients who do not require vaccination also helps finding out carriers and infected patients who require treatment [66].

Screening in the high endemic countries can be done by a single test of anti HBc antibody alone. This single test can detect individuals with current or past infection but fails to differentiate between recovered patients and carriers. A combination of the tests for HBs antigen and anti HBs antibody can also be used for screening.

This will in addition allow the identification of persons who are carriers and they can be followed and treated.

DOSE REGIMEN

Engerix B and Recombivax HB are the two yeast derived recombinant hepatitis B vaccines available. While EngerixB contains 20 mcg HBsAg/mL, Recombivax HB contains 10 mcg HBsAg/mL.

Three different manufacturers produce mammalian cell derived vaccines containing pre S epitopes. GenHevac B contains 20 ug/dose of vaccine and BioHepB/SciBVac contains 2.5 to 10 ug/dose and AG3 contains 10 to 20 ug/dose of the vaccine. The vaccination schedule for both mammalian cell derived and yeast derived vaccines is same in most countries. Three doses are given at one and six months apart from the first dose. In case of infants born to mothers with unknown or known positive HBsAg status, the 1st dose of vaccine has to be given within 12 hours after birth which should be followed by the 2^{nd}dose at one to two months and the 3rd dose at six months. The early administration of the vaccine confers early immunity for the neonates who have a risk of perinatal infection and in addition has a higher rate of onetime completion of vaccination series. For infants who are born to HBs antigen negative mothers, the 1st dose can be given before hospital discharge. The 2nd dose can be given at 1 to 2 months and the third dose at 6 to 18 months [64].

Vaccines should be administered by deep intramuscular route preferably deltoid in adults and vastuslateralis in infants and children. The deposition of vaccine in the adipose tissue especially if administered in gluteal region, results in a lesser seroconversion rate.

Longer intervals in between the given doses do not alter the final concentration of antibody levels. But the protection usually may not be attained until the recommended number of doses is completed. Hence, an interruption in the schedule does not necessarily require restarting the schedule. If the second dose has not been given it can be administered as early as possible and the third dose can be given after two months from the second dose. If the third dose is not given it can be given when possible. Protective anti HBs antibody titers in some may be achieved after 1 or 2 doses. But, completion of the entire course is recommended to maximize the titers of anti HBs antibodies and the duration of time the protection lasts.

EFICACY OF VACCINE

The hepatitis B surface antibody formation at an arbitrarily defined titres of >10mIU/mL is taken as a positive immune response to the vaccine. Studies are suggestive of the fact thatvaccinees with antiHBstiter above 10mIU/mL are protected. In a study it was concluded that acute infection rates were seven times more when these titers decreased below 10 mIU/mL.

Using this cut off of >10 mIU/mL antiHepatitis B antibody levels to be a positive response, the seroconversion frequency is around 95 % in healthy adults. This decreases as the age advances and is around 86 percent in the fourth decade and is around 47percent in the sixth decade [67].

The response to vaccine is a bit lower in men, obese individuals and smokers. The response is markedly less in those who have cirrhosis or chronic kidney disease. Patients who have undergone organ transplant, those having celiac disease and immunocompromised individuals have a low response. In patients on regular dialysis, the production of antibodies after the recombinant vaccines is around 50 to 60 % only yet risk of acquiring infection is 70 percent less in vaccinated individuals compared to the non-vaccinated.

TESTING POST VACCINATION STATUS

Routine testing after vaccination is not usually recommended as the response rate to vaccine is around 95 percent. The exceptions to this are patients on regular hemodialysis, health care workers and people at high risk such as kids born to the mothers who are carriers and sexual partners of the carriers.

The testing is performed 1 – 2 months after finishing the vaccination series. In case of kids born to HBs antigen positive mothers the testing is done at 9 to 15 months. Non responders are repeated a complete three dose series and it is successful in around 50 to 70 percent patients. Retesting for response should be done after second series of vaccination. If the person is not responding to the second dose also they have to be tested for HBsAg.

DURATION OF PROTECTION

It has been estimated that the protection against hepatitis B may persist up to 22 years after completing the primary vaccination series although the titers of anti HBs usually decreases with time. Booster doses are usually not recommended though there are exceptions. Although the anti HBs levels may not be in the detectable levels there is a reported protection from the disease. This may be due to anamnestic response due the memory T cell priming when challenged. This is evident from the fact that anti HBs titers increases rapidly after a booster dose in these individuals. A spontaneous rise in these titers along with seroconversion of anti HBc which is indicative of a rapid immune response to naturally occurring infection.

The durability of this anamnestic response is not completely understood. A variable proportion of the vaccinated persons over a period of time lose both the protective antibody titers and the anamnestic response.

There are various studies giving variable rates of protectiveness and anamnestic response.

Two studies involving adolescents who completed three doses beginning from birth gave a 48 to 70 percent anamnestic response after receiving a booster dose of vaccine.

In one more study involving high school children vaccinated at infancy immunity at 15 to 18 years was 90 percent [68, 69].

In a study done on Alaskan natives the protective levels of anti HBs was found in 87 percent after 22 years from vaccination [70].

One more study involving college students found that around 25 percent of those having undetectable levels of anti HBs antibodies and who were given the vaccine as infants did not mount an anamnestic response to a booster dose of vaccine[71].

As for now there is no strong evidence recommending booster doses of hepatitis B vaccine. There are ongoing studies in this field which may come out with results which would clear the doubts regarding booster doses.

Since the need for booster doses is not clear, giving a booster dose varies from country to country. Where some countries recommend the giving of boosters there are others which do not. However evaluating for anti HBs antibody titers and giving

booster dose of vaccine is recommended in patients on chronic dialysis and health care workers and at risk groups.

ADVERSE REACTIONS

Adverse reactions are reported in less than 25 percent of vaccinees. The most common being soreness over the injection site. 1 to 3 percent may have mild fever, headache, malaise, myalgia and joint pain. There are no serious adverse effects reported and the vaccine is safe during pregnancy with no teratogenic effect.

NON RESPONDERS AND THEIR MANAGEMENT

There are three types of non-responders to the vaccine [65]

- The first group of non-responders have an underlying medical disorder such as a chronic renal failure or immunosuppressed individuals. In these patients the response can be improved by doubling the dose of vaccine or by administering the vaccine through intradermal route. In patients who are to undergo dialysis a dose of vaccine is given before commencement of dialysis.

- The next group consists of individuals who are healthy but there is a genetically determined lack of response to vaccination. There may be a lack of a dominant response gene which controls the production of anti HBs. But the presence of the same haplotypes in persons with good response is suggestive of the fact that multiple factors may be responsible.

- The third group does no respond due to technical errors. This may include intragluteal injection or any inappropriate storage conditions.

The current recommendation is to give one or more additional dose of the vaccine to all healthy non responders. Only 15 to 25 percent respond adequately to a single additional dose but this may go upto 50 percent after three additional doses. So it is better to repeat three doses in case of non-responders. If still non-responsive additional doses may not benefit. These patients in addition should be tested for HBsAg.

Immunologically hyporesponsive patients may benefit from a newer vaccine which contains an adjuvant called immunostimulatory phosphorothioate oligodeoxy ribonucleotide in addition to HBsAg (HBVISS).

VACCINE INDUCED HBV S ESCAPE MUTANTS

In some infants hepatitis B infection occured despite of getting adequate serological response to vaccination [72]. In these infants HBV S gene substitution mutations have been identified in codon 145 “a” determinant. Such cases have been reported in China, Taiwan, Singapore, Italy, Japan and Africa. These mutations tend to decrease HBsAg to anti HBs binding and hence result in “escape” infection. This type of mutation has also been seen in patients developing recurrent HBV infection after liver transplantation despite of receiving HBIg prophylaxis.

The absence of these mutations in maternal carriers is suggestive of the fact that immune pressure (vaccine/HBIg) was responsible for mutation. Also the presence of the mutation in individuals who did not receive HBIg indicates that giving the vaccine alone may induce mutations. There are concerns regarding the increase in the incidence of escape mutations over time but studies show no decrease in vaccine efficacy and not much increase in HB S mutant incidence in pediatric population.

However continued monitoring is necessary and there is a need to develop newer vaccines.

IMMUNISATION SCHEDULE

The Indian Academy of Pediatrics recommends giving three primary doses of hepatitis B vaccine. The academy also recommends giving the first dose at birth to reduce the perinatal transmission rates. The best schedule recommended by the committee is the birth – 6 week – 6 month schedule of vaccine [72].

Though the above schedule is preferred, other options without increasing the number of visits in the existing national immunization schedule are also provided. These include

- At Birth, 6th and 14th week
- 6th, 10th and 14th week
- Birth, 6th week and 6th month
- Birth, 6th week, 10th week and 14th week

The committee does not recommend boosters for any of the schedules as of now. The routine testing of anti HBs levels one month after completing the three dose series is recommended for babies of HBs antigen positive mothers, health care workers and people with co morbidities. If they have not seroconverted repeating the three doses is adviced.

As far as the national immunization schedule followed by the government of India, the first dose of hepatitis B is given at birth for all institutional deliveries. Three other

primary doses are given at 6, 10 and 14 weeks along with DPT and HiB. In eight states it is given as Pentaxim, a combination vaccine with DPT and HiB.

Table 3 :The National immunization schedule

National Immunization Schedule for Infants, Children and Pregnant Women				
Vaccine	**When to give**	**Dose**	**Route**	**Site**
For Pregnant Women				
TT-1	Early in pregnancy	0.5 ml	Intra-muscular	Upper Arm
TT-2	4 weeks after TT-1*	0.5 ml	Intra-muscular	Upper Arm
TT- Booster	If received 2 TT doses in a pregnancy within last 3 yrs*	0.5 ml	Intra-muscular	Upper Arm
For Infants				
BCG	At birth or as early as possible till one year of age	0.1ml (0.05ml till 1mth age)	Intra-dermal	Left Upper Arm
Hepatitis B	At birth or as early as possible within 24 hours	0.5 ml	Intra-muscular	Antero-lateral side of mid-thigh
OPV-0	At birth or as early as possible within the first 15 days	2 drops	Oral	Oral
OPV 1,2 & 3	At 6 weeks, 10 weeks & 14 weeks	2 drops	Oral	Oral
DPT 1,2 & 3	At 6 weeks 10 weeks & 14 weeks	0.5 ml	Intra-muscular	Antero-lateral side of mid-thigh
Hep B 1, 2 & 3	At 6 weeks 10 weeks & 14 weeks	0.5 ml	Intra-muscular	Antero-lateral side of mid-thigh
Measles	9 completed months-12 months.	0.5 ml	Sub-cutaneous	Right upper Arm
Vitamin-A (1stdose)	At 9 months with measles	1 ml (1 lakh IU)	Oral	Oral
For Children				
DPT booster	16-24 months	0.5 ml	Intra-muscular	Antero-lateral side of mid-thigh
Measles 2nd dose	16-24 months	0.5 ml	Sub-cutaneous	Right upper Arm
OPV Booster	16-24 months	2 drops	Oral	Oral
Japanese Encephalitis**	16-24 months	0.5 ml	Sub-cutaneous	Left Upper Arm
Vitamin-A***				
(2nd to 9th dose)	16 months. Then, one dose every 6 months up to the age of 5 years.	2ml (2 lakh IU)	Oral	Oral
DPT Booster	5-6 years	0.5 ml.	Intra-muscular	Upper Arm
TT	10 years & 16 years	0.5 ml	Intra-muscular	Upper Arm

*Give TT-2 or Booster doses before 36 weeks of pregnancy. However, give these even if more than 36 weeks have passed. Give TT to a woman in labour, if she has not previously received TT.

** JE Vaccine, in select endemic districts after the campaign.

*** The 2nd to 9th doses of Vitamin A can be administered to children 1-5 years old during biannual rounds, in collaboration with ICDS.

NEW DEVELOPMENTS

Combination vaccines

The hepatitis B vaccine has been combined successfully with other vaccines without decreasing its efficacy. It has been combined with diphtheria, acellular pertussis, tetanus and hemophilus influenza vaccine. Combinations with Hepatitis A vaccine and with an inactivated polio vaccine also are available. The combination vaccines have the advantage of lesser number of injections, lesser hospital visits and better compliance.

Pre S vaccines

The HBV gene encodes for the three envelope proteins - the large S, the middle S and the small S proteins. All of the three have immunogenic B and T cell epitopes. The addition of pre S2 region to the routinely used vaccines did not improve the response rates. But in case of non-responders booster doses with vaccines containing S, pre S1and pre S2 regions induced a higher response compared to the conventionally used vaccines. These vaccines produced a better response rate of 71 percent after four doses in patients having chronic renal disease most of whom were on dialysis.

As for now these vaccines are licensed and are available inWestern Europe, Israel and in some Asian countries.

Single dose vaccines

A single dose hepatitis B vaccine prepared by encapsulating HBsAg in microparticles which are prepared from polylactidecoglycolide and polylactidepolymers. Particles of two different sizes, a <10 micron small particle and a 10 to 100 micron large particle are obtained. The vaccine contains 80 percent of these encapsulated microparticles. Upon inoculation the differently sized particles, the encapsulated and unencapsulated HBsAg are released at different times mimicking multiple dosing. Animal studies have found a good result but is yet to be tested in human beings.

Oral vaccines

The gene coding for HBsAg is incorporated in virulent recombinant salmonella strain and is administered orally. This salmonella in the gut produces HBsAg and this is presented to the mucosal macrophages which start producing anti HBs. Concerns regarding the reversal of virulence of the salmonella pose hinderance to the development of this vaccine.

Recently genetically modified bananas and potatoes which express HBsAg have been developed.

Inhaled vaccines

An inhaled vaccine of hepatitis B that is made with tetradecyl betamaltoside elicited a good response in rats by intratracheal administration. A promising response for an inhaled vaccine has been obtained in phase 1 study in humans.

DNA vaccines

These vaccines contain exposed DNA plasmids with HBV S gene. These vaccines after given through intramuscular route express HBsAg in the muscle cells and there is intracellular production of HBsAg. This stimulates anti HBs production. The HBsAg is degraded within the cells of muscles to peptides and these peptides are expressed on the surface of cells along with class 1 HLA molecules. This stimulates cytotoxic T cell production. The protective efficacy has been demonstrated in chimpanzees and data from human trials are yet to come.

ENHANCING IMMUNOGENICITY

The non-response rate of the conventional vaccines can be reduced by a number of methods

Intradermal inoculation

Inoculation of vaccine through intradermal route is supposed to be more immunogenic than intramuscular route. Though there are technical difficulties in administering the vaccine through intradermal route studies have shown intradermal

injections to have greater initial response and a better response in patients on hemodialysis and patients not having adequate response to initial vaccination.

New adjuvants

The immune response to hepatitis B vaccination can be augmented by administering along with interferon alpha, interferon gamma, or interleukin2. Discordant data have also been reported regarding this. Initial studies have shown vaccines that have incorporated potent immunogenic adjuvants like non alum based monophosphoryl lipid A and MF59 can induce higher levels of antiHBs. Further studies are required it this aspect.

Another vaccine in which HBsAg was combined with an adjuvant having 3'deacylatedmonophosphoryl lipid A along with alum had enhanced immunogenicity compared to the conventional vaccines. In a study after completing three doses of vaccine, the response rate for the adjuvant vaccine was 98 percent and among those who received the conventional vaccine it was 68 percent.

This vaccine is licensed and approved in Europe for patients more than 15 years of age having chronic renal insufficiency.

CHEMILUMINESCENCE METHOD FOR DETECTING ANTI HBs

The samples obtained from the subjects for this study were analyzed by the chemiluminescence method by the Abott Architect analyzer.

Figure 7 :Abottchemiluminescence analyzer

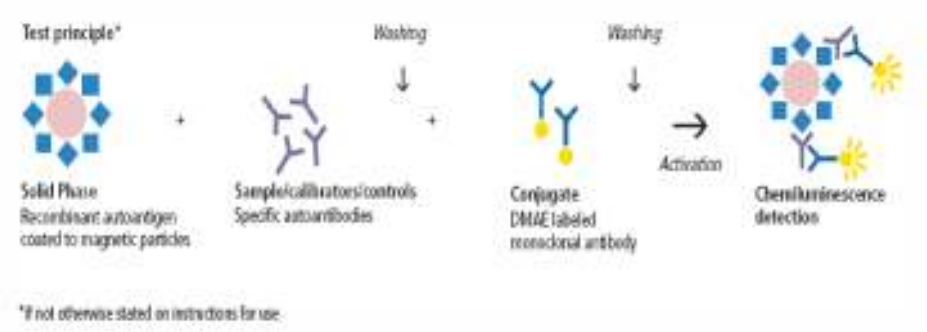
Chemiluminescence Kit (CLIA Kit) is a kit that is highly sensitive and uses the enzyme Streptavidin-HRPand the substrate reagent that is used is an enhanced ECL chemiluminecent system. The assay process of this Kit is as follows.

To begin with, the samples to be tested are added to the black opaque micro plates that are coated with antigens/antibodies.

Next the horseradish peroxidase complex is made to conjugate with the specific antigen/antibody that was added. The ingredients that did not react are then washed away.

Thirdly the Luminol chemiluminescent substrate is to be added into the microwells, and after that the photon counter reader scans the relative luminosity values (RLU).

Figure 8 : Principle of chemiluminescence kit



Chemiluminescent detection method technology is supposed to be a most advanced diagnostic methodasit has a very high sensitivity and there is no possible radioactivecontamination.

Table 4 : Advantages of CLIA kit over ELISA

Characteristic	CLIA Kit	ELISA Kit
High sensitivity	Minimum detection range levels at 10-18mol/L, important implication for early diagnosis of diseases	Minimum detection range levels at 10-9 mol/L
linear range	RLU values show a linear relationship within 4-6 orders of magnitude	Optical density(OD) values show a linear relationship within 2 orders of magnitude
Sample dilution	Sample with high concentration could be detected with stock sample to avoid the deviation in dilution	Sample with high concentration should be diluted before detection, and this might cause some deviation
Dilution fold of standard curve	3-4 fold dilutions series, the range of standard curve is quite wide, and the highest detectable concentration is 10000 times higher than the lowest detectable concentration	2 fold dilutions series, and the highest detectable concentration is 128 times higher than the lowest detectable concentration
Sample volume	50 ul of sample was utilized in some CLIA Kit	100 ul of sample was utilized in regular sandwich ELISA Kit

MATERIALS AND METHODS

STUDY DESIGN

Open labeled prospective study

STUDY POPULATION

Children aged 10 – 15 years attending to PSG hospital both in patients and out patients, who have record of immunization for hepatitis B in the infancy period.

MATERIAL & METHODS

Children aged 10 to 15 years who have received three hepatitis B vaccination doses before one year of age and who have records of the same are recruited for the study. Records are verified for the immunization status and confirmed. After getting proper consent for the study from the parent and obtaining assent from the children, 3 ml of blood sample is taken and levels of anti HBs levels determined by chemiluminescence immuno assay technique.

An anti-HBs level of ≥ 10 mIU/mL is considered to be a protective antibody level. Children with values < 10 mIU/ mL are considered to have inadequate antibody titers.

SAMPLE SIZE

30

DURATION OF STUDY

The study was conducted for a period of one year from September 2014 to August 2015.

INCLUSION CRITERIA

Children aged 10 – 15 years attending to hospital

Both in-patient and out-patient, who have record of immunisation for hepatitis B in the infancy period

EXCLUSION CRITERIA

History of hepatitis B infection

Hepatitis B infection in the mother

Patients having chronic renal failure

Children who are on chronic dialysis

Any transfusion history

Current use of steroids

Immunocompromised individuals

RESULTS

Out of the 30 subjects included in this study 14 were males and 16 were females (figure 9 and table 6). As for the age distribution 7 were between 10 and 11 years, 6 were between 11 and 12 years, 4 were between 12 and 13 years, 4 were between 13 and 14 years, another 5 between 14 and 15 years and 4 were between 15 and 16 years (figure 10 and table 7). The mean age was 12.2 years. Of the 30 subjects, 9 had anti HBs levels greater than 10mIU/mL (30%) out of which 5 had titers between 10 and 100 mIU/mL. These 30% are considered to be having protective titers. The rest 21 have titers less than 10mIU/mL and are considered to have inadequate protective antibody levels (70%). One subject had a titer of 0mIU/mL. Hence among the study population of 30 children aged 10 to 15 years who were vaccinated for hepatitis B during infancy, only 30% had protective titers of anti HBs antibody (figure 12). Of the 9 subjects who had protective levels of anti HBs antibody, 4 were males and 5 were females (figure 13). Of the protected 1 was in the age group on 10 – 11 years, 4 between 11 – 12 years, one between 12 – 13 years, 2 between 14 – 15 years and one was between 15 – 16 years (figure 14). The sex and the age between 10 and 15 years had no statistical significance on the levels of anti HBs.

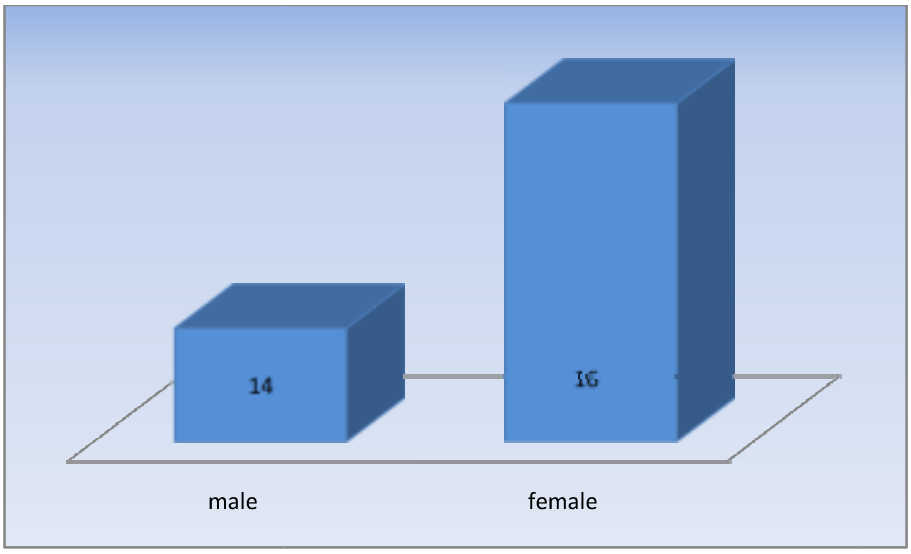
Table 5 :Sex wise distribution of subjects and anti HBs titers

SUBJECTS			ANTI HBs >10mIU/mL	ANTI HBs <10mIU/mL
TOTAL 30	MALE	14	4	10
	FEMALE	16	5	11

Table 6 :Age wise distribution of subjects and anti HBs titers

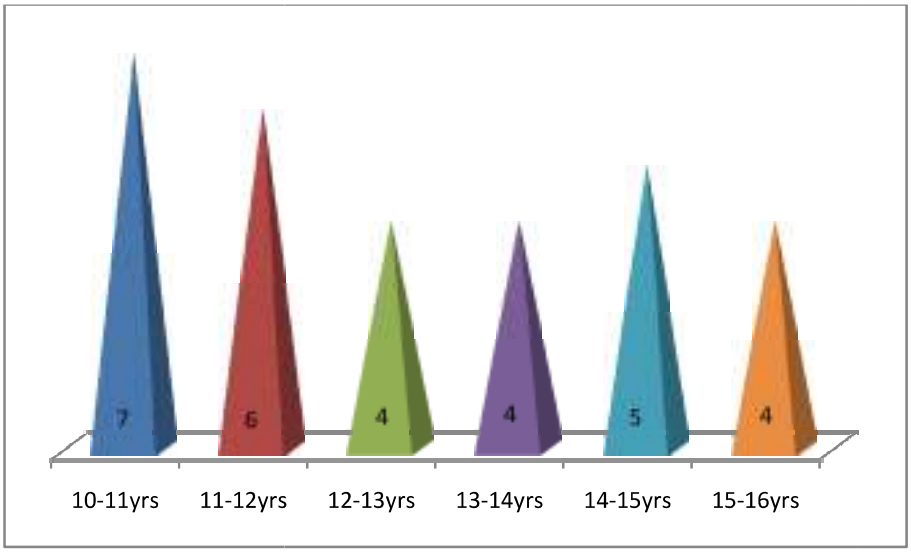
SUBJECTS			ANTI HBs >10mIU/Ml	ANTI HBs <10mIU/Ml
TOTAL 30	10-11 YEARS	7	1	6
	11-12 YEARS	6	4	2
	12-13 YEARS	4	1	3
	13-14 YEARS	4	0	4
	14-15 YEARS	5	2	3
	15-16 YEARS	4	1	3

Figure 9 : Sex distribution among subjects



Total subjects – 30 out of which males – 14 and females – 16

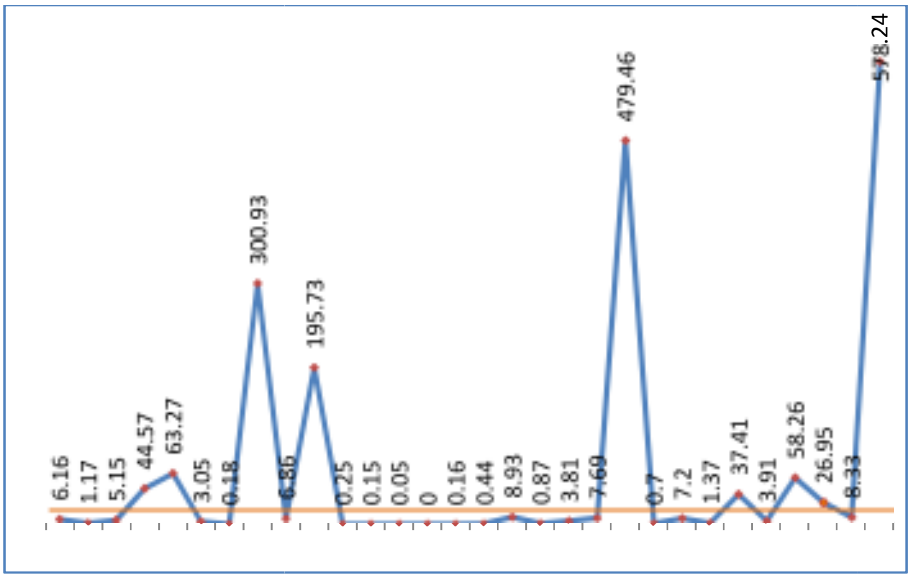
Figure 10 : Age distribution among subjects



10 - 11yrs – 7, 11 - 12yrs – 6, 12 - 13yrs – 4,

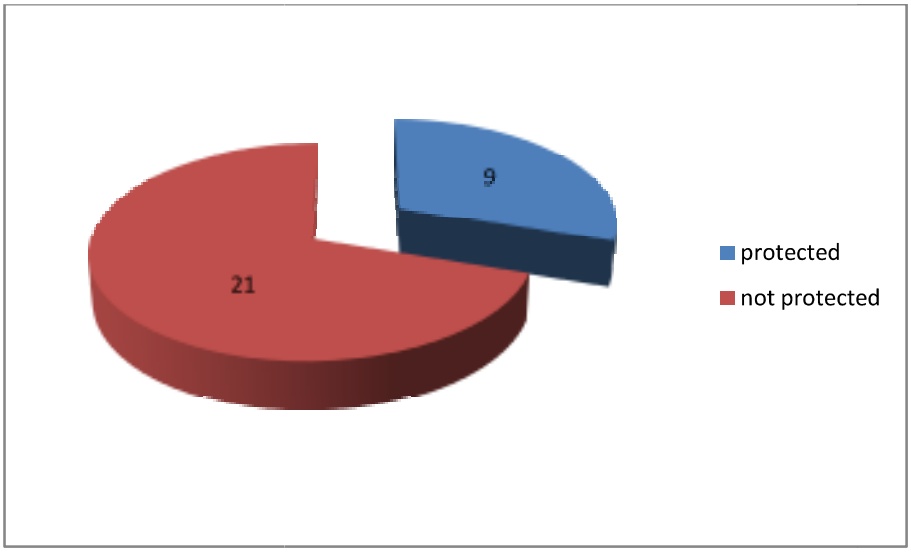
13 - 14yrs – 4, 14 - 15yrs – 5 and 15 - 16yrs – 4

Figure 11 : Anti HBs titers among the subjects



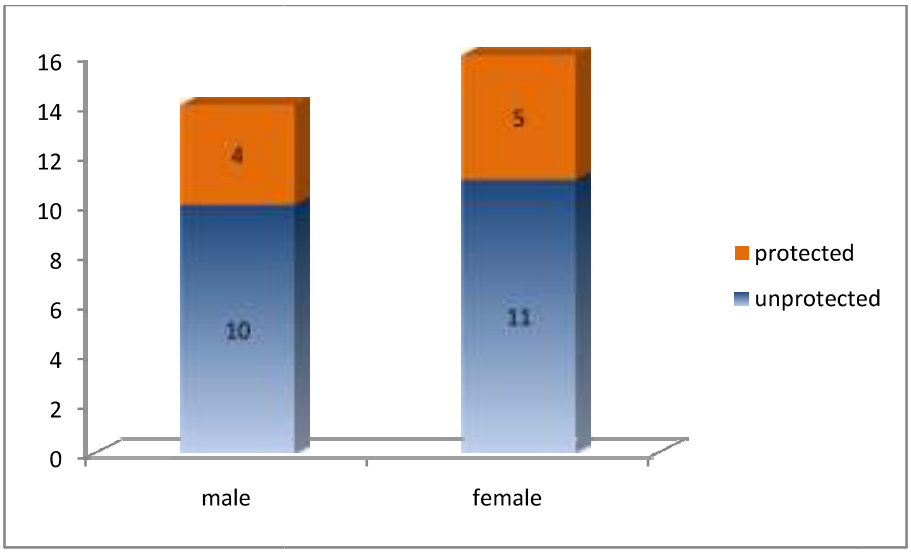
—10 mIU/mL mark (protective level)

Figure 12 : Protective levels of anti HBs antibody titers



Protective levels of anti HBs antibody (>10mIU/mL) – 9 out of 30 (30%)

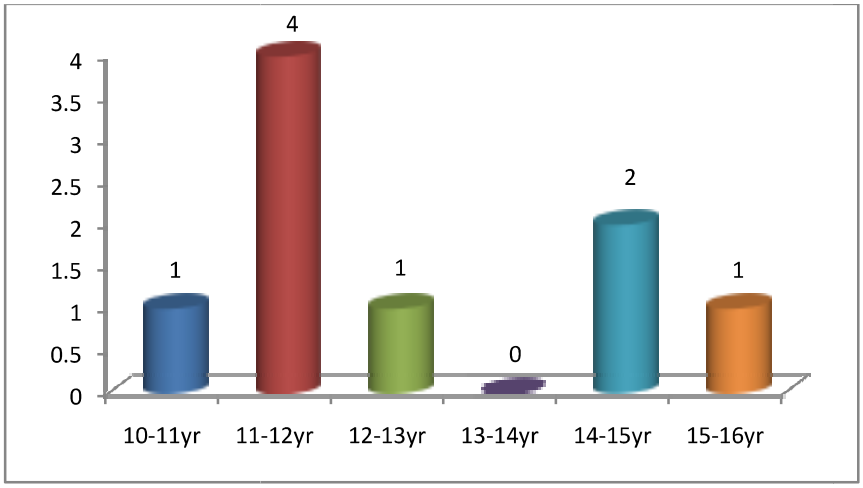
Figure 13 :Sex wise distribution of protective anti HBs levels



Out of 14 males 4 had anti HBs antibody levels >10mIU/mL (28.57%)

Out of 16 females 5 had anti HBs antibody levels >10mIU/mL (31.25%)

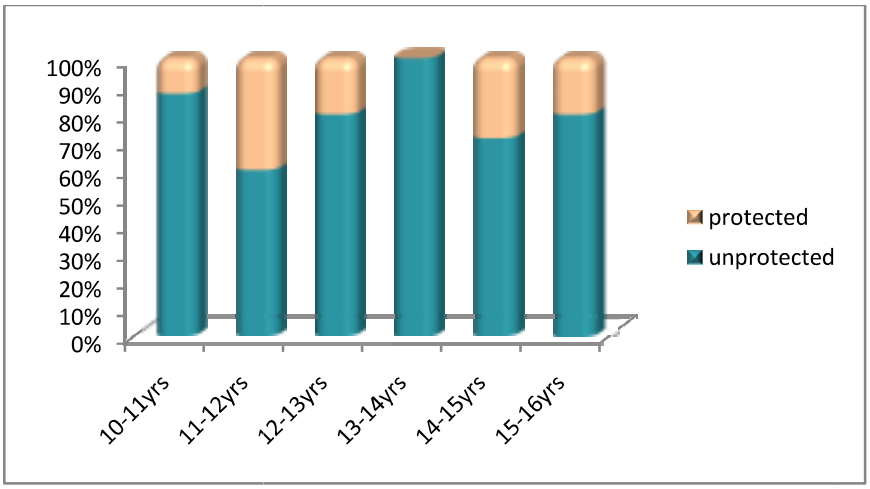
Figure 14 :Age wise distribution of protective anti HBs titers



The number of subjects in different ages with anti HBs levels >10 mIU/mL

10 – 11 yrs-1, 11-12 yrs – 4, 12 – 13 yrs-1, 13 -14 yrs -0, 14-15 yrs-2, 15-16 yrs-1

Figure 15 : Age wise protection percentage



There is no significant reduction in immunity levels with advancement of age

The age wise percentage of subjects protected are

10-11yrs –14.2%, 11-12yrs –66.6%, 12-13yrs –25%,

13-14yrs –0%, 14-15yrs –40%, 15-16yrs –25%

DISCUSSION

This study aimed at finding out the protectiveness against hepatitis B infection 10 years after complete vaccination and whether there is a need for further booster doses. Though there are only a very few studies available in this regard, none has come out from India. The studies from various regions have found out a variable percentage of protection by estimating the anti HBs levels in the serum. Since the vaccine failure has a genetic determinant also, it is necessary that we know the protective levels of anti HBs in Indian children ten years after completing the three doses of hepatitis B vaccine.

Our study has found that only 30% of the children who have completed the three doses of vaccine at infancy have protective titers of anti HBs antibodies after 10 years of age. This is lower than the percentage of protection found in many studies.

A study conducted in Ahvaz city in Iran by Reza Norouzirad et al evaluated the anti HBs levels in 840 healthy individuals in the age group between 1 and 18 years who were vaccinated at infancy. The percentage of study group who had protective levels of anti HBs (>10mIU/mL) 1 year after vaccination was 90%. The average number of subjects having protective levels of antibody titers between 1 and 5 years was 72.1% which declined to 40.1% in the age group between 11 and 15 years. A significant statistical correlation was attained between age and anti HBs levels [73].

In a study among 420 healthy children aged 16 to 19 years in Houston metropolitan area by Amy B. Middleman et al, it was found that only 24% of the

children had anti HBs levels in the protective range >10mIU/mL. But this study further checked the anamnestic response by giving one booster dose of hepatitis B vaccine and re checking antibody titers. 92% of children in the study group developed protective antibody titers after receiving one booster dose of the hepatitis B vaccine. This study thus concluded that though the antibody levels were less, there was an intact immune memory which was responsible for the rapid response after receiving the booster dose of vaccination. The study also compared to groups of vaccinees – one in whom the first dose of hepatitis B vaccine was given within 7 days and another group in whom the first dose was given after one month and found statistically significant variation in the baseline anti HBs antibody values between the two groups with the early vaccinated group having decreased titers [74]. This is of interest to us because many of our children are given the first dose of hepatitis B vaccination at birth.

In a study in Italy by E.Spada et al, 571 teenagers were included and their anti HBs levels were tested at 10 and 17 years of age. 60.3% of the subjects had protective antibody levels to HBsAg. Out of the remaining 227 subjects 199 were administered a booster dose of hepatitis B vaccine. The percentage of the subjects having protective antibody levels was 72.9% at 17 years which included the persons who received the booster dose of the vaccine. Out of the 155 subjects who had anti HBs levels <10mIU/mL, 96 were given booster dose of the vaccine to which all except two responded. The remaining two subjects received also responded after three doses of the vaccine. This study also concludes that though the antibody titers may be less, the immune response is good after a booster dose [75].

A study was conducted by James W. Keck et al involving 104 out of 389 children from the youth hepatitis B protection study. In this study children who were boosted with an additional dose of hepatitis B vaccine at 5 to 7 years in one group and between 10 to 13 years in another two groups were taken and the anti HBs levels were assessed 7 to 9 years after the booster dose. It was found that even after a booster dose anti HBs levels was greater than 10mIU/mL in only 42% of the individuals. This study also concludes pre booster and 4 week post booster levels of anti HBs were significant predictors of vaccine immunity at follow up [76].

HivaSaffar et al conducted a study in Iran in which 176 individuals vaccinated for hepatitis B at infancy were administered one booster dose of hepatitis B vaccine at 20 years of age. Pre booster anti HBs values and the pre and post booster cytokine values were taken. It was found that only 42.6% had protective pre booster values of anti HBs of more than 10mIU/mL. Among the non-protected the cytokine values showed a two fold increase after a booster dose in around 80% of individuals confirming preserved cell mediate immunity [77].

The effect of giving three doses of boosters was studied in a study conducted in China by Paul K.S. Chan which found that out of 212 students aged 17 to 23 years only 18.9% had protective anti HBs titers. One month after a single booster this went up to 85.5% and after three booster doses this number reached 100% with 97.1% having anti HBs titers above 100mIU/mL. This study demonstrates a declining immune memory in a substantial group of individuals and the requirement of three

doses of booster vaccination to achieve protective antibody titers. This study recommends pre booster testing of anti HBs titers and vaccinate accordingly [78].

These studies give a wide range of protection against hepatitis B infection. The 30% protective efficacy obtained in our study is line with that obtained in many of the studies. Some of the studies have assessed the immune memory by giving a booster dose of hepatitis B vaccine and found that most of the subjects had a good immune memory and responded very well to a single booster dose of the vaccine.

Interestingly there have been two studies from Alaska which give a considerably lower percentage of anamnestic response. This is particularly significant since these two studies involved a significant number of American Indians along with Alaskan natives.

One of these studies included 37 children who were vaccinated with all three primary doses of hepatitis B before 1 year and who had documented anti HBs titers >10mIU/mL before 18 months of age. The anti HBs levels of these subjects were done at 14 to 15 years of age and it was found that only 5% had protective levels of anti HBs. The subjects with anti HBs antibody levels <10mIU/mL were boosted with a dose of vaccine and the titers of anti HBs rechecked after 15 days. Anamnestic response to booster dose was only 51% [79].

In another study by Samandari et al there were three groups of participants. In the first group there were 74 adolescents within the age group of 11.7 to 14.9 years those who were vaccinated with plasma derived hepatitis B vaccine the first dose of the vaccine being given before 1 week of age. The second and third group had

subjects vaccinated with recombinant vaccine in the age group between 10 to 14.7 years and children in the age group 5 to 7 years respectively. These subjects were given one booster dose of hepatitis B vaccine and the rate of anamnestic response was assessed 2 weeks after giving the booster dose. It was found that the anamnestic response was inversely related with the age. The anamnestic response to hepatitis B vaccine dropped from 97% at 5 years to 60% at 14 years of age [80].

The difference in the protective titer levels of antibodies in different populations studied, the waning of anamnestic response with age in certain populations and lack of studies in the Indian population warrants studies in this regard. Our study gives a protective level of antibodies to HBs in only 30% of the studied subjects who completed three doses of hepatitis vaccine before one year of age. A larger study with a larger sample and also studies assessing the anamnestic response is required in Indian population to find out the necessity of booster doses in the general Indian population.

The study also exposes the need for testing anti HBs titers and the need for booster doses of vaccination. If not possible in all, at least in the more vulnerable groups like health care workers and contacts of HBsAg positive individuals should be screened for anti HBs levels.

LIMITATIONS OF THE STUDY

1. Small number of subjects.
2. HBs Ag antigens and core antibodies have not been done for financial reasons.
3. Anamnestic response to a booster dose was not tested due to financial reasons.

CONCLUSION

1. 70% of the children aged 10 – 15 years who were vaccinated for hepatitis B before one year of age have anti HBs titers in the sub protective range.

2. Out of the 30% who have anti HBs titers in the protective range, majority are between 10 and 100 mIU/mL.

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ABBREVIATIONS

HBV	-	Hepatitis B virus
HCV	-	Hepatitis C virus
HDV	-	Hepatitis D virus
HBeAg	-	Hepatitis B envelope antigen
HBsAg	-	Hepatitis B surface antigen
HBcAg	-	Hepatitis B core antigen
Anti HBs	-	Antibodies to hepatitis B surface antigen
Anti HBc	-	Antibodies to Hepatitis B core antigen
HCC	-	Hepatocellular carcinoma
HIV	-	Human immuno deficiency virus
HBIg	-	Hepatitis B immunoglobulin.
PT	-	Prothrombin time
ALT	-	Alanine transaminase
AST	-	Aspartate transaminase
WHO	-	World Health Organization
DNA	-	Deoxy ribonucleic acid
RNA	-	Ribo nucleic acid
ORFs	-	Open reading frames
NTCP	-	Sodium taurocholate co transporting polypeptide
ccc DNA	-	Covalently closed circular DNA
PCR	-	Polymerisedchainraction
INR	-	International normalized ratio
AFP	-	Alphafetoprotein
WBC	-	White blood cell

PATIENT INFORMATION SHEET

Name :

Age :

Sex:

Address:

Contact number:

Whether he / she has been immunized against hepatitis B	yes / no
If yes how many doses were given and at what age?	_____
History of jaundice in the past	yes / no
History of jaundice in the mother during pregnancy	yes / no
History of recent fever	yes / no
Any history of transfusion	yes / no
Any history of current medications	yes / no
If yes, specify	_____
Any current / chronic illness	yes / no
If yes, specify	_____

Institutional Human Ethics Committee
PSG Institute of Medical Sciences and Research, Coimbatore

Assent to be in a Research Study
For children between 7-18 years old

Why are we meeting with you?

We want to tell you about something we are doing called a research study. A research study is when doctors collect a lot of information to learn more about something related to health and disease.Dr.Kumaraguruand some other doctors are doing a study to learn more about **Prevalence of seropositivity against hepatitis B virus in children aged 10 to 15 years who have been immunized against hepatitis B in infancy.** After we tell you about it, we will ask if you'd like to be in this study or not.

Why are we doing this study?

We want to find out the protection against Hepatitis B virus for which you have been immunized before you were one year of age.
So we are getting information and blood samples from lots of boys and girls like you.
In the whole study, there will be about 30 children.

What will happen to you if you are in this study?

Only if you agree, you will be included in the study.

1. 3 ml of blood will be taken for investigation.
2. This sample will be used for laboratory testing.

Will this study hurt?

No this won't hurt you. You may experience a slight discomfort while blood samples are being taken.

Will you get better if you are in this study?

No, this study won't make you feel better or get well. But the doctors might find out something that will help you and other children like you later.

Will everybody come to know about my condition? (Confidentiality)

We will not tell other people that you are in this research and we won't share information about you to anyone who does not work in the research study

Is this bad or dangerous for me? (Risks involved)

No there is no risks involved

Do I get anything for being in the research?

No

Will you tell me the results?

Yes we will tell u the results whenever you want to know. These results will also be published in a book for research purpose. But we will not reveal your name in it.

Do you have any questions?

You can ask questions any time. You can ask now. You can ask later. You can talk to me or you can talk to someone else.

Do you have to be in this study?

You will be included in the study only if you agree. No one will force you to participate in the study. If you don't want to be in this study, just tell us. And, remember, you can say yes now and change your mind later. It's up to you. This will not affect in any way your future treatment in this hospital.

Who can I talk to or ask questions to?

I am giving you my mobile number. You are free to clarify your doubts at any time. You can also discuss with your parents or teachers at any time regarding this study.

SIGNATURE OF PERSON CONDUCTING ASSENT DISCUSSION

I have explained the study to _____ in a language he/she can understand, and the child has agreed to be in the study.

Dr. Kumaraguru.R

Date :

Place : Coimbatore

CERTIFICATE OF ASSENT

I have read this information (or had the information read to me). I have had my questions answered and know that I can ask questions later if I have them.

I agree to take part in the research.

Signature :

Name :

Date :

Place : Coimbatore

IF ILLITERATE

I have witnessed the accurate reading of the assent form to the child, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Signature of witness :

Name of the witness :

Thumb print of participant :

Date :

Place : Coimbatore

PSG Institute of Medical Science and Research, Coimbatore
Institutional Human Ethics Committee

INFORMED CONSENT FORMAT FOR RESEARCH PROJECTS

(strike off items that are not applicable)

I **Dr.Kumaraguru.R** with I.D.No.**703157**, am carrying out a study on the topic **Prevalence of seropositivity against hepatitis B virus in children aged 10 to 15 years who have been immunized against hepatitis B in infancy** as part of my research project in the department of Paediatrics, PSG IMS & R.

My research guide are Dr. A.M. Vijayalakshmi and Dr. Jayavardhana and this study is being sponsored by PSG IMS & R.

Since very less is known about the long term efficacy of Hepatitis B vaccination, this study aims at finding out the efficacy of vaccine given before 1 year, at the age of 10 to 15 years.

Children between the ages of 10 to 15 who have a record of hepatitis B vaccination in the infant period will be recruited to the study. The expected sample size is 35 for this study.

2 – 3 ml of blood taken solely for the purpose of this research will be taken from the participant and the levels of anti HBs antigen levels will be determined.

The advantage of participation in this study is that you will come to know about your child's protectiveness against hepatitis B virus.

There are no risks involved in this study except that your child may have a slight discomfort when the blood samples are being taken.

We request you to kindly cooperate with us in this study. We propose collect background information and other relevant details related to this study.

We will be carrying out:
Initial interview for around 10 minutes where you will be asked to fill a proforma.
2 – 3 ml of blood will then be collected and sent for determining anti HBs antigen levels

Data collected will be stored for a period of five years and confidentiality of the data will be maintained strictly. Under no circumstances shall we reveal the identity of the respondent or their families to anyone. The information that we collect shall be used for approved research purposes only.

We will not use the data and the samples collected as part of another study.

If you are uncomfortable in answering any of our questions during the course of the interview / biological sample collection, **you have the right to withdraw from the interview / study**

at anytime. You have the freedom to withdraw from the study at any point of time. Kindly be assured that your refusal to participate or withdrawal at any stage, if you so decide, will not result in any form of compromise or discrimination in the services offered nor would it attract any penalty. You will continue to have access to the regular services offered to a patient. You will **NOT** be paid any remuneration for the time you spend with us for this interview / study. You will be informed about any significant new findings - including adverse events, if any, – whether directly related to you or to other participants of this study, developed during the course of this research which may relate to your willingness to continue participation.

CONSENT

The above information regarding the study, has been read by me/ read to me, and has been explained to me by the investigator/s. Having understood the same, I hereby give my consent to them to interview me. I am affixing my signature / left thumb impression to indicate my consent and willingness to participate in this study (i.e., willingly abide by the project requirements).

Signature / Left thumb impression of the Study Volunteer / Legal Representative:

Signature of the Interviewer with date: Witness:

Date :
Place : Coimbatore

Contact number of PI : 9841991276

Contact number of Ethics Committee Office: 0422 2570170 Extn.: 5818

மனித உரிமை கோட்பாடுகள் குழு

PSG மருத்துவக் கல்லூரி மற்றும் மருத்துவமனை, கோவை

இந்த ஆராய்ச்சி 10 முதல் 15 வயது நிரம்பிய குழந்தைகளிடம் செய்யப்படுகிறது.

நாம் எதற்கு சந்திக்கிறோம் ?

நாங்கள் அராய்ச்சி படிப்பைப் பற்றிக் கூற விரும்புகிறோம். ஆராய்ச்சி படிப்பு என்பது மருத்துவர்கள் ஒரு குறிப்பிட்ட மருத்துவ தகவல்களை சேகரித்து அதைப் பற்றி தெரிந்து கொள்வது.

நான் Dr. ரா. குமரகுரு, ஒன்று வயதிற்குள் மஞ்சல் காமாலை பி தடுப்பூசி போட்ட குழந்தைகளுக்கு 10–15 வயதில் எதிர்ப்பு சத்து உள்ளதா என்பதை கண்டறிகிறேன்.

எதற்காக இந்தப் பரிசோதனை ?

இதன் மூலம், பத்து முதல் பதினைந்து வயதுக்குட்பட்ட குழந்தைகளில் மஞ்சல் காமாலை பி எதிர்ப்பு சத்து உள்ளதா என்று ஆராயப்படும். இதற்காக நிறைய தகவல்களை ஆண் மற்றும் பெண் குழந்தைகளிடம் இருந்து இரத்தம் பரிசேதனைக்குப் பெறுகிறோம்.

இந்த ஆய்வில் மொத்தமாக 30 குழந்தைகள் உள்ளனர்.

உங்களின் பரிசோதனையின் முடிவுகள் வேறு யாருக்கும் செய்யப்பட மாட்டாது.

உங்களுக்கு மட்டுமே தெரியப்படுத்தப்படும் இந்த பரிசோதனையில் உங்களுக்கு சந்தேகம் இருந்தால் Dr. ரா. குமரகுரு – **98419 91276** தொடர்புக்கொள்ளவும்.

இந்த பரிசோதனையில் நீங்கள் பங்கு கொள்ள வேண்டும் என்ற கட்டாயம் இல்லை.

உங்களுக்கு விருப்பம் இல்லை என்றால் இதில் இருந்து எப்போதும் வேண்டுமானாலும் விலகிக் கொள்ளலாம்.

இதனால் இந்த மருத்துவமனையின் எந்த சலுகைகளும் குறைக்கப்பட மாட்டாது.

Dr. ரா. குமரகுரு ஆகிய நான் இந்த சோதனையைப் பற்றி அனைத்து தகவல்களையும், குழந்தைகளின் பெற்றோர்களுக்கு தமிழில் கூறியுள்ளேன்.

குழந்தையின் பெற்றோர் அதை நன்கு புரிந்து பிறகு இதற்கு சம்மதம் தெரிவித்துள்ளனர்.

உங்களிடம் 2–3 மில்லி இரத்தம் எடுத்து சோதனை செய்யப்படும்.

இந்த பரிசோதனை வழக்கமாக செய்யப்படும் பரிசோதனை மட்டுமே.

இந்த பரிசோதனையின் முடிவை மட்டுமே எனது ஆய்வுக்கு எடுத்துக்கொள்வேன்.

ஆய்வுக்குட்பட்டவரின் கையொப்பம் **ஆய்வாளரின்**

கையொப்பம்

தேதி :

பகுதி – 2

சம்மத சான்று

நான் இந்தக் தகவலைப் படித்தேன் (அல்லது) தகவலை படிக்கக் கேட்டேன் எனது கேள்விகளுக்குப் பதில் அளிக்கப்பட்டது மற்றும் கேள்விகள் எதுவும் இருந்தால் அவைகளை வருங்காலத்தில் கேட்கலாம் என்பதும் எனக்குத் தெரியும்.

நான் இந்த ஆராய்ச்சியில் பங்கேற்க சம்மதிக்கிறேன்.

அல்லது

நான் இந்த ஆராய்ச்சியில் பங்கேற்க விருப்பம் இல்லை மற்றும் கீழே சம்மதம் என்று கையொப்பம் செய்யவில்லை. ..... .

(குழந்தை / மைனர் / இனிசியல் செய்யப்பட்டுள்ளது)

குழந்தை மட்டும் சம்மதம் தெரிவித்தால்

குழந்தையின் பெயர் :

குழந்தையின் கையெழுத்து :

தேதி :

படிப்பறிவு இல்லாதவராக இருந்தால் படிப்பறிவு உள்ள சாட்சி கையொப்பம் செய்ய வேண்டும் (முடிந்தால் இந்த நபர் கலந்து கொள்பவரால் தேர்ந்தெடுக்கப்பட வேண்டும். இந்த நபர் பெற்றோராக இருக்கக்கூடாது. மற்றும் அவருக்கு ஆராய்ச்சி குழுவிடம் எந்த தொடர்பும் இருக்கக்கூடாது).

நான் சம்மதம் படிவத்தை குழந்தையிடம் சரியாக படித்துக் காண்பித்ததைப் பார்த்தேன். மற்றும் அந்த நபருக்கு கேள்விகள் கேட்க வாய்ப்பு இருந்தது. அந்த நபர் தனது சம்மதத்தை முழு மனதுடன் கொடுத்தார் என்பதை நான் உறுதி கூறுகிறேன்.

சாட்சிகள் :

சாட்சியின் கையெழுத்து :

தேதி :

கலந்து கொள்பவரின் இடது
பெருவிரல் ரேகை

ஆராய்ச்சி செய்பவரின்

பெயர் : டாக்டர் இரா. குமரகுரு

கைபேசி எண். 98419 91276

பெற்றோரின் ஒப்புதல் படிவம்

ஆய்வின் தலைப்பு:

ஒன்று மொழிக்குள் மூன்றாம் மொழி திணிப்பு
குழந்தைகளுக்கு 10-15 மொழிகள் எதிர்பு சந்து உள்ளதா என்பதை கண்டறிதல்.

ஆய்வு மேற்கொள்பவர்: இரா. முருகு

துறை : குழந்தைகள் நலத்துறை

இந்த ஆய்வில் நான் ஒன்று மொழிக்குள் மூன்றாம் மொழி திணிப்பு குழந்தைகளுக்கு 10-15 மொழிகள் எதிர்பு சந்து உள்ளதா என்பதை குறித்து ஆராய்ச்சி செய்கிறேன். உங்கள் குழந்தையை இந்த ஆய்வில் ஈடுபடுத்துமாறு கேட்டுக்கொள்கிறோம்.

என் பெயர் இரா. முருகு நான் பி எஸ் ஜி மருத்துவமனையில் முதுகலை மாணவராகயிருக்கிறேன். இந்த ஆய்வில் இந்த ஆய்விற்கு குறைந்தது 35 குழந்தைகள் பங்கேற்க வேண்டுமென்பதால் உங்களுடைய குழந்தையை இந்த ஆய்வில் பங்கேற்க அனுமதிக்குமாறு கேட்டுக்கொள்ளப்படுகிறது. நீங்கள் அப்படி அனுமதிப்பதினால்தான் உங்களுடைய குழந்தைக்கு 2-3 மில்லி இரத்தம் எடுத்து அதில் மூன்றாம் மொழி எதிர்பு சந்து உள்ளதா என்பது கண்டறியப்படும். இந்த குறிப்புகள் இரகசியமாக வைக்கப்படும். பிற்காலத்தில் உங்களுடைய விருப்பத்தின்பேரில் மட்டுமே பிறருக்கு தெரியப்படுத்தப்படும். நீங்கள் உங்கள் குழந்தையை இந்த ஆய்வில் பங்குபெற அனுமதிப்பதினால் உங்களுக்கும் பி எஸ் ஜி மருத்துவமனைக்கும் உள்ள இணைப்பு எந்த விதத்திலும் பாதிக்கப்படாது. உங்களுக்கு ஏதேனும் கேள்விகள் அல்லது சந்தேகங்கள் இந்த ஆய்வைப் பற்றி இருந்தால் 98419 91276 என்ற தொலைபேசி எண்ணிற்கு அழைக்கலாம். நீங்கள் இந்த படிவத்தில் இரண்டு கையொப்பம் உங்கள் குழந்தையை இந்த ஆய்வில் பங்கேற்க அனுமதிக்கிறீர் மற்றும் நீங்கள் இந்த படிவத்தை முழுமையாக படித்து புரிந்து கொண்டீர்கள் என்பதை உறுதிசெய்கிறது. நீங்கள் எதிர்காலத்தில் இந்த ஆய்விலிருந்து விலகிக் கொள்ள நினைத்தால் எவ்விதக் தெரியப்படுத்தலாம். நீங்கள் எப்பொழுது வேண்டுமானாலும் இந்த ஆய்விலிருந்து விலகிக் கொள்ளலாம். இதனால் உங்களுக்கு எவ்விதமான சிக்கலும் எந்த விதத்திலும் ஏற்படாது.

குழந்தையின் பெயர்:

பெற்றோர் . காப்பாளர் கையொப்பம் :

தேதி :

ஆய்வாளரின் கையொப்பம் :

MASTER CHART

S.NO.	IP/OP NUMBER	AGE	SEX	ANTI HBs TITER (mIU/mL)	PROTECTION
1	i14028786	15	m	6.16	N
2	i14028886	11	f	1.17	N
3	i14030678	13	m	5.15	N
4	i14034784	12	m	44.57	Y
5	i14034064	11	m	63.27	Y
6	i14036625	12	f	3.05	N
7	i15000779	15	m	0.18	N
8	i15002459	11	m	300.93	Y
9	i15002166	15	f	6.86	N
10	i15002896	15	f	195.73	Y
11	i15007308	10	m	0.25	N
12	i15009255	13	m	0.15	N
13	o15021256	10	m	0.05	N
14	i15011925	14	f	0	N
15	o15027552	10	f	0.16	N
16	i15012462	10	f	0.44	N
17	i15012903	11	f	8.93	N
18	i15014670	14	m	0.87	N
19	i15017061	12	m	3.81	N
20	i15017311	13	f	7.69	N
21	i15017559	11	f	479.46	Y
22	o05028610	10	m	0.7	N
23	i15020296	13	f	7.2	N
24	i15024357	12	f	1.37	N
25	i15024551	14	f	37.41	Y
26	i15024361	14	f	3.91	N
27	i15024390	10	f	58.26	Y
28	i15025033	14	m	26.95	Y
29	i15025321	10	m	8.33	N
30	i15025609	11	f	578.24	Y

M- Male F - Female N - No Y - Yes